National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

REGULATION OF T CELL RESPONSES BY PEPTIDES OF THE T CELL RECEPTOR β CHAIN VARIABLE REGION

by

DONNA M. MACNEIL ©

A thesis submitted to the faculty of graduate studies and research in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

MEDICAL SCIENCES (IMMUNOLOGY)

EDMONTON, ALBERTA

FALL 1992

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-77370-7

Canada

University of Alberta
Edmonton
Canada T6G 2H7

Department of Immunology
Faculty of Medicine
860 Medical Sciences Building, Telephone (403) 492-2275
Fax (403) 492-0368

02 October 1992

Dr. F.S. Chia, Dean
Faculty of Graduate Studies and Research
2-8 University Hall
University of Alberta

Dear Dr. Chia:

We grant permission to Donna MacNeil to reproduce the following material in her thesis.

MacNeil, D., Fraga, E. and Singh, B.: *Inhibition of Superantigen Recognition by Peptides of the Variable Regions of the T cell Receptor β Chain*, Eur. J. Immunol. 22: 937-941, 1992.

Sincerely,

Bhagirath Singh

Bhagirath Singh, Ph.D.
Professor of Immunology

Ester Fraga
Technologist

Ester Fraga

BS/hj

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR:	DONNA M. MACNEIL
TITLE OF THESIS:	REGULATION OF T CELL RESPONSES BY PEPTIDES OF THE T CELL RECEPTOR β CHAIN VARIABLE REGION
DEGREE:	DOCTOR OF PHILOSOPHY
YEAR THIS DEGREE GRANTED:	FALL 1992

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Donna MacNeil

Donna M. MacNeil

10710 - 80th Avenue. Apt. 101
Edmonton, Alberta, TGE 1V8

Date: October 8, 1992

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled

Regulation of T cell responses by peptides of the T cell receptor β chain variable region

submitted by Donna M. MacNeil in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Sciences (Immunology)

Bhagirath Singh (Supervisor)

Kevin Kane (Co-supervisor)

Larry Guilbert

Linda Pilarski

Walter Maksymowych

Ellen Heber-Katz

Date: October 1, 1992

DEDICATION

To my husband

Thomas Otto Wilhelm Mueller

and to my parents

William Ralph MacNeil and Dorothy Louise MacNeil

with love and gratitude

ABSTRACT

We have investigated the regulation of T cell responses by peptides of the variable region of the TCR. We have shown that Vβ-peptides can affect T cell responses *in vitro*. Several Vβ6-peptides can partially inhibit the response of Vβ6$^+$ T cell hybridomas to a superantigen, Mls-1^a. The sequences of the inhibitory peptides delineate the region of the TCR which interacts with Mls-1^a. This region was found to be outside the antigen binding site of the TCR.

To determine the effect of Vβ-peptides on T cell responses *in vivo*, we first characterized the T cell response to Vβ-peptides in several strains of mice. We found most Vβ-peptides to be immunogenic in the strains of mice tested. Responsiveness correlated well with MHC haplotype but not to the Mls phenotypes. Endogenous superantigens, such as the loci of the Mls system, cause the deletion of peripheral T cells bearing certain Vβ gene segments. Thus expression of a particular Vβ did not induce tolerance to peptides of that Vβ. The T cell response to Vβ6-peptides was found to be primarily by CD4$^+$, class II restricted T cells. Several of the Vβ6-peptides could also induce antibody responses in BALB/c mice, but the anti-Vβ6-peptide antisera could not bind to intact TCR of Vβ6$^+$ T cell hybridomas. We also observed that one peptide could not induce an antibody response in spite of a good T cell proliferative response *in vitro*. This peptide appears to preferentially induce the

Th1 subset of T cells as demonstrated by the production of IL-2 and IFNγ by these T cells.

In vivo, Vβ-peptides can also down-regulate T cell responses. Pre-immunization with Vβ6-peptides led to a decreased proliferative response to antigens, Mls-1^a and $(EYA)_5$, which stimulate mainly $V\beta6^+$ T cells. However, peptides from similar regions of the Vβ3 gene segment could not protect recipient mice from the transfer of the T cell mediated autoimmune disease, Type I diabetes. Our findings are consistent with the proposal that peptides of the variable region of the TCR have a role in the regulation of T cell responses.

ACKNOWLEDGEMENTS

I thank my supervisor Dr. Bhagirath Singh for his advice and his guidance of my graduate program and for his support of my thesis work. I thank the members of my supervisory and examining committees, Drs. Kevin Kane, Larry Guilbert, Linda Pilarski, Walter Maksymowych, and Ellen Heber-Katz for their advice and suggestions, as well as for their critical review of my thesis.

I am grateful to Rucy Vergidis and Dong-er Gong for their patience in showing me the ropes. I am also grateful to Dr. Soledad Sawada for her support and friendship over the years since I began my studies in this department. I thank Jana Lauzon for lending me her ear on so many occasions, for her assistance and advice, technical and otherwise. I also thank Ann Burrell for her friendship, technical advice, as well as many exciting matches of squash. I thank Babita Agrawal for many thought provoking discussions and her invaluable comradeship. I also thank Tom Dillon for his assistance and countless cups of coffee. I thank Ester Fraga for synthesizing all the peptides necessary for my thesis work, and for answering all my naive questions concerning chemistry. I am indebted to all the graduate students, members of the office staff, and others of the Department of Immunology for making this department an enjoyable place to work.

I am especially grateful to my parents who taught me to work hard always and who never wavered in their support of me. I also sincerely thank my husband, Thomas Mueller, who taught me to believe in myself.

Lastly, I thank Dr. Erwin Diener for introducing me to Immunology.

TABLE OF CONTENTS

CHAPTER PAGE

I. Introduction

T cell recognition of antigen 1
T cell recognition of superantigen 11
Thymic selection 15
Tolerance 21
Autoimmunity 28
Regulation of T cell responses 35
Project and Rationale 37
Bibliography 41

II. Inhibition of superantigen recognition by peptides of the variable region of the T cell receptor β chain (Paper 1)

Introduction 71
Materials and methods 73
Results and discussion 76
Bibliography 86

III. Characterization of murine T cell response to peptides of self T cell receptor β chains (Paper 2)

Introduction 90
Materials and methods 93
Results 99
Discussion 105
Bibliography 123

IV. Effect of pre-immunization with self peptides of the TCR on the T cell response to superantigen, peptide antigen, and on the transfer of diabetes in NOD mice (Paper 3)
Introduction 129
Materials and methods 132
Results 136
Discussion 140
Bibliography 150

V. General discussion and further studies 154
Bibliography 164

LIST OF TABLES

TABLE	DESCRIPTION	PAGE
2.1	Mls-1^a specificity of T cell hybridomas	81
2.2	Sequences of $V_\beta 6$ peptides	82
3.1	Sequences of $V\beta$ peptides	113
3.2	Response to peptides of the T cell receptor in several strains of mice	114
3.3	Frequency of $V\beta6^+$ and $V\beta8^+$ lymph node cells after *in vitro* stimulation with antigen	116
4.1	Sequences of peptides used in this study	144
4.2	Response by primed NOD mice to peptides of $V\beta3$ T cell receptor	145

LIST OF FIGURES

FIGURE	DESCRIPTION	PAGE
2.1	Inhibition of recognition of Mls-1^a by $V\beta6^+$ T cell hybridomas with $V\beta6$ peptides	83
2.2	$V\beta6$ peptides cannot inhibit recognition of Mls-1^a by the $V\beta8^+$ T cell hybridoma, B9	84
2.3	$V\beta6$ peptides cannot inhibit recognition of peptide antigen, $(EYA)_5$ at 20 μM, by the $V\beta6^+$ T cell hybridoma, B11	85
3.1	Antibody response to Vβ6 peptides by BALB/C mice	117
3.2(a)	Anti-Vβ6 peptide sera do not bind to the intact Vβ6 TCR	118
(b)	BALB/c mice with anti-Vβ6(1-20) sera retain $V\beta6^+$ peripheral T cells	118
3.3	Production of IL-2 by T cells stimulated with Vβ6 peptides	119
3.4	Production of IFNγ by T cells stimulated with Vβ6 peptides	120
3.5	Inhibition of T cell response to Vβ6 peptides with mAb to class II MHC	121
3.6	Inhibition of I-A^d restricted T cell hybridoma, A.1.1, by Vβ6 peptides at various concentrations..	122

4.1 Effect of immunization with Vβ6 peptides on BALB/c mice response to Mls-1^a 146
4.2 Effect of immunization with Vβ6 peptides on CBA/CaJ mice response to Mls-1^a 147
4.3 Effect of immunization with Vβ6 peptides on BALB/c mice response to $(EYA)_5$ 148
4.4 Effect of splenocytes from NOD mice primed with Vβ3 peptides on the transfer of diabetes in NOD mice 149

ABBREVIATIONS

Ab	antibody
APC	antigen presenting cell
ATP	adenosine triphosphate
B cell	bone marrow derived lymphocyte
BFA	brefeldin A
C	constant
CDR	complementarity determining region
CFA	complete Freund's adjuvant
c.p.m.	counts per minute
CTL	cytotoxic T lymphocyte(s)
D	diversity
DTH	delayed type hypersensitivity
EAE	experimental autoimmune encephalomyelitis
EAU	experimental autoimmune uveitis
ER	endoplasmic reticulum
FγG	fowl gamma-globulin
H-2	mouse major histocompatibility complex
Hb	hemoglobin
HEL	hen egg lysozyme
HLA	human major histocompatibility complex
[^{3}H]dThd	[methyl-^{3}H]-thymidine
H-Y	male specific antigen
Ii	invariant chain

Ig	immunoglobulin
IFNγ	interferon-gamma
IL	interleukin
J	joining
kBq	kilobecquerel
kD	kilodalton
Kd	equilibrium disassociation constant
LMP	low molecular weight protein(s)
M	molar concentration (mol/litre)
mAb	monoclonal antibody
MBP	myelin basic protein
MHC	major histocompatibility complex
MLR	mixed lymphocyte reaction
Mls	minor lymphocyte stimulatory locus
Mtv	mammory tumour virus
PPD	purified protein derivative
SE	staphalococcal enterotoxin
SEA	staphalococcal enterotoxin A
SEB	staphalococcal enterotoxin B
SEC	staphalococcal enterotoxin C
T cell	thymus derived lymphocyte
TCR	T cell receptor
TGFβ	T cell growth factor-beta
Th	helper T lymphocyte(s)
TSST-1	toxic shock syndrome toxin 1
V	variable

CHAPTER I

Introduction

Antigen specific recognition is one of the distinguishing features of adaptive immunity of vertebrates. The specificity of the immune response is due to the clonotypic receptors of specialized lymphocytes, B cells and T cells. The antigen specific receptors of B cells, immunoglobulins (Ig), which are expressed on the cell surface and secreted by B cells, can interact with soluble, intact antigen. The T cell receptor for antigen (TCR), expressed only as a transmembrane receptor, recognizes peptide fragments of protein antigen in the context of antigen presenting molecules, the major histocompatibility (MHC) molecules.

T cell recognition of antigen

Structure of TCR and MHC molecules

The T cell receptor for antigen (TCR) is a clonotypic, disulfide linked heterodimer (1, 2) non-covalently associated with CD3, a monomorphic complex of several subunits (3). The transmembrane heterodimer is composed of α and β (expressed by the majority of peripheral T cells) or γ and δ glycoproteins (expressed by a minority of peripheral T cells, 4). The heterodimer confers both antigen specificity as well as MHC restriction to the T cell (5, 6). Although the tertiary structure of the TCR has not been empirically

determined, it has been inferred by comparison to the X-ray crystallographic analyses of immunoglobulins, based on the homology of the TCR to the immunoglobulin molecules of B cells. Both glycoprotein chains of the TCR heterodimer posses two characteristic immunoglobulin-like domains formed by intrachain disulfide bonds: a variable N-terminal domain and a constant C-terminal domain (7). The variable domain is encoded by one each of several variable (V), diversity (D, in the cases of β and δ chains), and joining (J) gene segments which recombine with a constant (C) gene segment to form a complete gene (8, 9). The Ig-domains of each TCR chain consist of two anti-parallel β-pleated sheets forming a β barrel. Loops connecting the β strands of the variable domain at the distal N-terminal of the molecule contain hypervariable regions of great diversity, which form the antigen binding site and interact with the antigen-MHC complex (10).

The CD3 complex, which is obligately but non-covalently associated with the TCR on the cell surface of T cells (3), is composed of several transmembrane glycosylated proteins: γ, δ (not to be confused with the TCR chains of the same name), two ε proteins, and either a ζζ or a ζη disulfide linked dimer (11). The CD3 complex is involved in signal transduction of the T cell upon activation but is not involved in the recognition of the antigen-MHC complex per se (12).

The molecules encoded by the major histocompatibility complex (MHC) were originally identified as transplantation antigens, blood group antigens, genes for susceptibility to tumor transplants, and Ia antigens (13). MHC molecules play a role in cellular interactions of the immune response by binding antigen (14) and presenting antigen to T cells. This requirement for recognition of antigen by T cells, is termed MHC restriction, ie. T cells recognize antigen presented by APC only when the T cell and the APC share MHC molecules (15). The MHC is highly polymorphic, being both multigenic and multiallelic (16); the MHC locus is named H-2 in the mouse and HLA in the human. Genes of the MHC are classified into at least three categories: class I and class II molecules, which present antigen to T cells, and class III molecules, which are serum proteins and some complement components and are not always considered to belong to the MHC. The class I loci are K, D, and L in the mouse (16), and A, B, and C in the human (17). The class II loci are I-A, I-E, and MaMb in the mouse (16, 18), and DP, DQ, DR, and DM in the human (17, 19). Class I molecules are composed of a transmembrane glycoprotein, the α chain, with three extracellular Ig-like domains, which is non-covalently associated with a smaller extracellular protein with one Ig-like domain, β_2-microglobulin. Class II molecules are composed of two transmembrane gylcoproteins, an α chain and a β chain, both of which have two extracellular Ig-like domains (13). In general, class I molecules present intracellular antigen to CD8 positive CTL and class II molecules present extracellular antigen to CD4 positive T helper

cells (20). CD8 and CD4 are transmembrane invariant molecules expressed by most T cells. CD8 and CD4 act as co-receptors in antigen recognition; they associate with an invariant portion of class I and class II molecules, respectively (21, 22), and are involved in signalling during activation of T cells (23).

The tertiary structure of class I MHC molecules has been determined by X-ray crystallography of several human class I molecules (24). The membrane proximal Ig-domain of the α chain, $\alpha 3$, is associated with β_2-microglobulin, forming a base on which sit the N-terminal Ig-like domains of the α chain, $\alpha 1$ and $\alpha 2$. The $\alpha 1$ and $\alpha 2$ domains form a unique structure, unlike the usual Ig-domains, consisting of a β pleated sheet lying underneath two α-helical segments. A space between the two α-helicies on top of the β sheet forms a groove, proposed to be the antigen binding site of class I. Consistent with this hypothesis is the fact that most of the polymorphic amino acids of the class I molecule important for T cell recognition are located in or adjacent to the putative antigen binding groove (25). Also, in the X-ray crystallography analysis, this groove was found to be occupied by an unknown peptide (24). To date, the tertiary structure of class II MHC molecules has not been empirically determined. It is assumed, based on amino acid sequence homology to class I, that class II molecules have a very similar structure with the antigen binding groove being formed by the distal Ig-like domains of the α and β chains.

Antigen processing

As was previously stated, T cells recognize antigen in the form of small peptides associated with MHC molecules. These peptides are produced from the degradation of proteins by antigen presenting cells (APC), a process termed antigen processing. It has been known for a decade that antigen processing is a time and temperature dependent process which requires an acidic intracellular compartment (26), and can include denaturation and proteolytic cleavage of the native antigen. (27). Antigen processing is a general term used to describe what are actually several intracellular mechanisms for producing peptide fragments. There are at least two major routes for antigen to travel before being presented by either class I or class II molecules. One major route of processing is taken by endogenous peptides presented by class I molecules (28, 29). These peptides are generated in the cytosol by a ubiquitin-dependent multisubunit proteasome, which has several peptidase activities at neutral pH. It may be that proteasomes are a class of enzymatic structures, one of which, the low-molecular-weight-proteins (LMP) (30), specializes in providing peptides to class I MHC molecules for presentation (31, 32). Several of the genes coding for subunits of LMP have been cloned and mapped to within the MHC in human (33) and in mouse (32). Peptides are then translocated across the endoplasmic reticular membrane by peptide transporters. Two genes coding for peptide transporters were originally identified by cloning and sequencing previously unsequenced regions within the MHC gene complex (34). They

share sequence homology to the ATP-binding cassette (ABC) superfamily of transporters (34). Transfection of these genes into mutant cell lines can restore class I expression and antigen presentation of viral antigens to T cells (35, 36). The two proteins likely form a heterodimer, which is located in the endoplasmic reticular membrane (37). Together, the structural and functional data are consistent with the proposed role of these proteins as transporters of peptides to class I MHC. Although the above described route of cytoplasmic proteins is probably the major source of peptides for presentation by class I molecules, a second source of peptides has been recently suggested. Signal peptides, cleaved from newly synthesized transmembrane and secreted proteins, have been extracted from class I molecules of a mutant cell line lacking functional peptide transporters (38, 39). Since signal peptides have been observed on cells with no defect in transporter genes (40), it is possible that signal peptides are a source of peptides even in "normal" cells.

Peptides derived from endogenously synthesized proteins, associate with the α-chain and β2-microglobulin. In fact, peptide association with class I may be necessary for the expression of a stable class I molecule on the cell surface (41). Inhibitors of protein synthesis can inhibit class I presentation of antigen, indicating newly synthesized class I molecules associate with antigen (41). Also, brefeldin A (BFA), which inhibits transport of molecules into the cis-Golgi, also inhibits antigen presentation by class I molecules

(42), indicating that antigen association to MHC molecules occurs in the endoplasmic reticulum (ER) or pre-Golgi compartment. From the ER, class I-peptide complexes traffic through the Golgi apparatus to the cell surface, as do most transmembrane molecules (41). In addition, antigen processing may take place on the cell surface by a serum protease, angiotensin-converting enzyme (ACE) (43). The peptide generated at the cell surface may occupy "empty" class I molecules or displace resident peptides from the binding groove of the class I molecules (44).

The second major route of antigen processing involves the degradation of exogenous proteins in an acidic compartment for presentation by class II molecules (28, 45). Exogenous antigen is endocytosed by specialized APC and undergoes proteolysis en route to the lysosomal compartment. As quickly as two minutes after endocytosis, antigen, class II, proteolytic enzymes and the invariant chain (Ii) can be observed in an early endosomal compartment (46). Other studies have shown antigen encounters class II molecules in a specialized late endosomal compartment (47). In either case, class II molecules are directed from the constitutive secretory pathway and enter the endocytic pathway. Because cyclohexamide and BFA can both inhibit antigen presentation by class II molecules (48), it has been concluded that newly synthesized class II molecules bind to peptides in this compartment. Further support for this conclusion is the finding that antigen associates with class II molecules which are inaccessible to neuraminidase treatment, ie.

nascent molecules (49). In this case, Ii may play an active role in directing the intracellular route taken by nascent class II molecules and in peptide binding. It is also possible that class II molecules can be recycled from the surface to the endocytic compartment where they exchange peptides (46) and that peptides derived from endogenous proteins may be presented by class II molecules (50, 51).

The peptides generated by antigen processing have been identified by using synthetic peptides to stimulate cloned T cells. In studies attempting to identify the minimum sequence necessary to induce immune responses (52), or stimulate cloned T cells (53, 54), the smallest peptide required varied from 7 to 15 amino acids. In more recent studies, the sequences of naturally processed peptides eluted from class I and class II molecules have been determined. Peptides eluted from class I molecules are 8 to 12 amino acids in length (40, 55, 56) and are shorter than those eluted from class II molecules, which vary from 12 to 17 amino acids long (57). In some cases a sequence motif could be identified from peptides binding to a particular class I or class II molecule, indicating that certain residues are important in peptide binding to the MHC antigen binding groove (56 - 58).

It has been proposed that the peptide in the antigen binding groove of the MHC molecule assumes an α-helical conformation (59, 60), but this model is not applicable to all peptides (61). The X-ray

crystallography pattern of the electron dense mass in the binding groove of one class I molecule is consistent with a nonomeric peptide in an extended conformation (62), however it is likely that the secondary structure assumed by the peptide is variable and is dependent on both the peptide sequence and the MHC molecule which binds the peptide.

Interaction of TCR, MHC, and antigen

As stated in a previous section, the antigen binding site of the TCR is comprised of the three distal loops of the β-barrel formed by the variable domains of the α and β chains. These loops correspond to highly variable complementarity determining regions, CDR1, CDR2, and CDR3. How these regions interact with antigen and MHC molecules has been a highly debatable topic. Various models have been proposed involving specific interactions between one chain or the other of the TCR and antigen and/or the MHC molecule (63). The most recent model proposed (64, 65) incorporates several aspects of the TCR-MHC-peptide interaction. In this model, peptide binds to the groove with specific residues interacting with the MHC molecule or the TCR, CDR1 and CDR2 of both the α and β chains interact with the α-helical regions of the antigen binding groove on the MHC molecule, and CDR3 of both the α and β chains interact with the peptide. Recent evidence from several studies favors this model of TCR-antigen-MHC interaction. In one study, mice transgenic for either the α or the β chain of a particular TCR heterodimer were immunized with substituted peptides of the

antigen recognized by that TCR (66). Then the endogenous α and β chains of the responding T cells were sequenced to identify specific residues which interact with the peptide residues. The most important residues for peptide interaction were located in the CDR3 loops of the α and β chains. In another study, T cell hybridomas were transfected with the wild-type β chain and specifically mutated α chains of a TCR (different from the one used in the previous study). Again, a substitution to the CDR3 of the α chain greatly altered the pattern of antigen recognition, consistent with the model that CDR3 contacts peptide (67). Also, in a fine specificity analysis of T cell clones, differences in antigen recognition were associated with sequences of the CDR3 in the α and β chains of the different TCRs (68). Thus, the CDR loops of the TCR have specific roles in antigen recognition and MHC restriction.

The affinity of this tertiary interaction has not been measured directly, but two independent studies have attempted to measure the affinity of the TCR for antigen and MHC molecules indirectly, and have arrived at similar conclusions; this interaction is very weak compared to antibody binding to specific ligands. In one study, soluble peptide-MHC complexes were used to compete with a specific monoclonal antibody for binding to the TCR (69). The affinity of the peptide-MHC complex for the TCR was estimated to have a K_d of 6×10^{-5} M, by using the affinity of the antibody for the TCR. In another study, soluble TCR was used to inhibit antigen-specific activation of T cell hybridomas (70). Comparing the

concentration of soluble TCR to that of antibody to MHC needed for 50% inhibition, the affinity of the TCR for the peptide-MHC complex was estimated to have a K_d of 5×10^{-6} M. Both estimates are much lower than the range of Kd for antibody binding to soluble antigen (10^{-8} to 10^{-10} M) (69). This low affinity of the TCR for the peptide-MHC complex implies that this interaction must contribute little to the adhesion reactions between T cells and APC, but this interaction is still the required event for activation of the T cell (71).

T cell recognition of superantigen

Definition of Superantigen

Superantigens are antigens which can stimulate a large fraction of T cells based on the TCR Vβ usage. The superantigens endogenous to mice were identified almost two decades ago by the observation that a strong primary mixed lymphocyte reaction (MLR) could be obtained between some pairs of mouse strains which had the same MHC haplotype (72). The antigens responsible for this stimulation were termed minor lymphocyte stimulating (Mls) determinants. Originally four Mls types were identified, but another rather confusing classification system has been developed based on the strength of stimulation in MLR assays among several strains of mice (73). In the newest system, the Mls phenotype is described by the genotype at three loci, named 1, 2, and 3, each with an "a" and a "b"

allele (74). Other endogenous superantigens have been identified which are not part of the Mls system (75, 76).

The strong stimulation in MLR between Mls disparate strains is due to the ability of Mls determinants to stimulate all T cells bearing a particular Vβ. The link between TCR Vβ use and reactivity was first identified between Vβ6 and Mls-1^a (77), but many more examples have been established between other Vβs and endogenous superantigens (77 - 81). Mouse strains carrying the stimulatory alleles of Mls loci do not have T cells bearing the appropriate Vβ in the peripheral spleen or lymph nodes (75, 77, 82).

The exogenous superantigens are bacterial products, such as Staphylococcal enterotoxins (SE), toxic shock syndrome toxin 1 (TSST-1) (83), and *Mycoplasma arthritidis* (MAM) (84), which share the mitogenic activity of Mls in that SE, TSST-1 and MAM stimulate T cells bearing a particular Vβ. For example, SEB stimulates murine T cells bearing Vβ3, Vβ7, Vβ8, or Vβ17, whereas MAM stimulates T cells bearing Vβ6, or Vβ8 (85). While the bacterial superantigens have been easily isolatable and identifiable proteins, Mls determinants and other endogenous superantigens have been elusive since their discovery (74). The putative genes encoding for some endogenous superantigens may be gene(s) of mouse mammary tumour viruses (Mtv) (86). This suggestion is based on strong correlations between expression of a particular Mtv-provirus and an endogenous superantigen. Transfection of the gene encoded

in the open reading frame of the 3' long terminal repeat of Mtv-2 (87) or of the milk borne C3H Mtv (88) resulted in the clonal deletion or *in vitro* stimulation of the appropriate T cell subset, respectively.

Superantigens differ from peptide antigens in many aspects. First, both the endogenous and exogenous superantigens do not exhibit the classical MHC restricted recognition of peptide antigens. Although class II molecules act as receptors for SE (89) and anti-class II antibodies can block stimulation of T cells by Mls or SE, most MHC haplotypes can present Mls or SE regardless of the MHC haplotype of the responding T cell (74, 83). In spite of the involvement of class II molecules, CD4 and CD8 are not necessary for the interaction between superantigens and T cells. CD4 negative CD8 positive ($CD4^-CD8^+$) T cells bearing TCR Vβ6 or Vβ8, can respond to Mls-1^a (90, 91), and $CD4^-$ T cell hybridomas with TCR Vβ8.1 are able to respond to SEA, SEB, or TSST-1 (92). Lastly, SE do not require processing for recognition by T cells (93), which is related to the manner of interaction between superantigens and TCR.

Interaction of TCR, MHC, and superantigen

An integral part of superantigens is their ability to stimulate most T cells based on TCR Vβ usage. Unlike that for peptide antigens, specificity for Mls-1^a can be transferred between T cell hybridomas with the β chain of the TCR only (94). Also, direct binding of SEA to

β chains of the TCR has been demonstrated (95, 96). This implies that superantigens interact only with the Vβ portion of the TCR. The site of interaction of the TCR Vβ with Mls-1^a and SEC has been identified by various means. Transfection of a chimeric β chain containing residues from position 67 to 77 of a TCR Vβ able to recognize SEC conferred recognition of SEC to the recipient T cell hybridoma (97). Site specific mutagenesis of TCR Vβ8.2 identified residues at positions 22, 70, and 71 as being important in recognition of Mls-1^a (98, 99). Peptides corresponding to amino acids 1 to 20 or 58 to 75 of TCR Vβ6 inhibited the recognition of Mls-1^a by Vβ6 bearing T cell hybridomas (Chapter 2, 100). All studies show that a region on the β-pleated sheet of Vβ, which is exposed to solvent and away from MHC-peptide or Vα interactions, binds to superantigen. The site of interaction between class II molecules and superantigens has been demonstrated for only SE and TSST-1, not for any of the endogenous superantigens. Peptides of the I-Aβ^d chain corresponding to the α-helix of the antigen binding groove blocked enterotoxin binding to class II molecules (83), and site directed mutagenesis of the α chain identified specific residues of the α-helix which were important in binding of SEA, specifically those residues which face outward away from the cleft of the antigen binding groove (101). TSST-1 also binds to the α-helix of the α and β chains of class II molecules (102). These results demonstrate that superantigens do not contact CDR3 of the TCR and enterotoxins do not bind MHC in the antigen binding groove.

Thymic selection

Stem cells from the bone marrow colonize the embryonic thymus and continue to do so throughout adult life (103). Stem cells entering the thymus bear few identified markers, but during ontogeny express CD4, CD8, then CD3 and TCR, as well as other differentiation molecules on the cell surface (104). Most of the T cells which exit from the thymus, loose expression of either CD4 or CD8 based on the specificity of the TCR, and expression of either determines restriction to class II or class I MHC molecules. These mature T cells have been positively selected for restriction to self MHC and negatively selected for tolerance to all self molecules. Although immature T cells bearing γδ TCR develop in the fetal thymus before αβ-expressing T cells (104), much of the work on positive and negative selection has been done with αβ-expressing T cells.

Positive Selection

The earliest demonstration of positive selection was the observation that MHC restriction was "learned" rather than genetically determined. The experiments demonstrating "learning" of MHC restriction involved irradiation bone marrow chimeras, in which T cell depleted-bone marrow cells of (P1 x P2)F1 progeny were injected into irradiated hosts of either parental strain. The F1 T cells acquire the MHC restriction of the host parental strain, not that of the F1 or second parental strain (105, 106). These results were

consistent with the selection of F1 T cells which were capable of recognizing antigen presented by the host type MHC molecules. Subsequent experiments using thymectomized mice, indicated that the MHC type expressed by the thymus determines the restriction of the T cells (107, 108). These experiments have the disadvantages that *in vivo* priming is required to determine MHC restriction, and that the lymphocytes are never 100% of donor origin. However, other studies have shown that positive selection by MHC molecules is required for normal thymocyte development.

Anti-class II monoclonal antibody (MAb) administered neonatally, prevents the development of $CD4^+8^-$ T cells (109) but not $CD4^-8^+$ (110). Similarly, injection of anti-class I MAb from birth prevents the development of $CD4^-8^+$ T cells (111). In addition, mice which lack the expression of any class I molecules, also lack $CD4^-8^+$ T cells (156), and mice, which lack class II molecules, have greatly reduced levels of $CD4^+8^-$ T cells compared to normal mice (113, 114).

Other studies have also overcome the drawbacks of the chimera studies using as tools, transgenic mice and clonotypic antibodies for TCRs of known specificity. Mice transgenic for a rearranged TCR β chain lacking the Vβ region, lacked $\alpha\beta^+$ thymocytes, and $CD4^+8^-$ and $CD4^-8^+$ thymocytes, confirming that positive selection by MHC interaction with TCR is required for normal development (115). In Mls-1^b mice which express I-E of the k or d haplotype, the frequency of $V\beta6^+$ T cells is higher than that of mice lacking I-E.

This has been interpreted to mean that, in the absence of Mls-1^a, which is preferentially presented by I-E^k or I-E^d, the V$\beta6^+$ TCR retains some affinity for I-E alone and is positively selected to a greater degree than in mice lacking I-E (116). Similarly, the frequency of V$\beta17a^+$ T cells is higher in mice of the H-2^q haplotype than that of mice of H-2^b. Since the frequency of V$\beta17a^+$ T cells in (q x b)F1 mice is also elevated, negative selection could not be the cause of the reduced frequency of V$\beta17a^+$ T cells in H-2^b mice, therefore positive selection is likely to account for the elevated frequency of V$\beta17a^+$ T cells in H-2^q and the F1 mice (117). Mice transgenic for a TCR specific for cytochrome c (cyt c) and I-E^k, have elevated $CD4^+$ T cells only when the mice express I-E^k (118). Mice of the H-2^b haplotype, which are transgenic for the same TCR, have elevated levels of $CD4^+8^+$ thymocytes but few $CD4^+8^-$ T cells, further supporting the requirement for positive selection in T cell development (119). Similarly, mature $CD8^+$ T cells develop in female mice transgenic for a TCR specific for the male (H-Y) antigen and D^b, only if the mice express the D^b molecule (120).

Although it has been established that the thymus is the most important site of positive selection during T cell development, there has been some debate over the origin of the thymocytes which control positive selection. There is much evidence to support radiation-resistant, epithelial cells of the thymus in imposing MHC restriction (106 - 108). Others contend that radiation-resistant, resident bone marrow cells of the thymus confer MHC restriction,

and that if these cells are rigorously removed with high levels of irradiation or pre-treatment of thymus grafts, T cells of bone marrow chimeras will acquire MHC restriction to the bone marrow donor haplotype (121). This debate appeared at a stand-still until the new technique of gene transfer was used to re-address this question. By using different promoters in construction of transgenes of the Eα chain, transgenic mice expressing I-E in different tissues have been generated (122). Positive selection of $V\beta6^+$ T cells occurred only in those transgenic mice in which I-E was expressed on the cortical epithelial cells of the thymus (123). The only caveat to these studies is that low levels of I-E, which are undetectable by immunohistology, may be expressed by other cells of the thymus. The possible mechanism of positive selection will be discussed together with that of negative selection in the following section.

Negative Selection

Negative selection, together with loss due to incorrectly rearranged TCR genes, is thought to account for the apparently vast cell death of immature T cells which occurs in the thymus during ontogeny (124). There is much evidence that negative selection in the thymus occurs via clonal deletion of self-reactive T cells. As previously mentioned, mice expressing endogenous superantigens (77, 82, 125) or mice injected neonatally with enterotoxin (126) delete T cells expressing the reactive Vβ. Mice expressing $I\text{-}E^k$

delete Vβ17a$^+$ T cells (75); Vβ17a$^+$ T cells from I-E$^-$ mice are stimulated by I-E^k and Eα^{k}Eβ^s in the absence of antigen (80). Interestingly, injection of anti-CD4 MAb blocked deletion of not only the CD4$^+$, but also the CD8$^+$ T cells (127, 128). In male mice transgenic for the TCR specific for H-Y antigen and D^b, there are very few CD4$^+$8$^+$ thymocytes (129), as well as fewer CD4$^+$ T cells in the lymph nodes. These results indicate that deletion of the developing T cells occurs when the immature T cells express both CD4 and CD8. As with positive selection, negative selection occurs only in the presence of the restricting MHC molecule (130). These results are consistent with above findings that deletion occurs at the CD4$^+$8$^+$ stage of T cell development, and requires interaction of the MHC molecule with the TCR. Recently, the demonstration of negative selection has been extended to γδ+ T cells specific for the Tla class I molecule (131).

The site of deletion in the thymus is most likely to occur in the cortico-medullary junction, since Vβ6$^+$ T cells are found in the cortex but not the medulla of Mls-1^a mice (132). Again, it is unclear which cell type is responsible for clonal deletion. Bone marrow derived cells in the thymus can induce deletion of autoreactive T cells (130, 133). Transgenic mice which express I-E^k only on epithelial cells of the thymus, have been reported to delete Vβ17a$^+$ T cells (122). On the other hand, bone marrow chimeras or thymus engrafted mice do not delete T cells reactive to autoantigens expressed only by the thymic epithelium (134 - 136).

The mechanism of negative selection appears to be the induction of apoptosis. Several studies show that signalling through the TCR of immature thymocytes (expressing low levels of CD3) by either exposure to anti-CD3 MAb (137) or antigen (138 - 140) can induce the immature thymocytes to die by apoptosis. This supports the "affinity model" of selection in which an immature T cell bearing a TCR with a strong affinity for self antigen will be deleted when it encounters that self antigen (141). In the affinity model, positive selection is the rescue from cell death of those immature T cells which have some (but not too strong) affinity for self-MHC molecules (141).

Rather than different affinities for self-MHC by the TCR resulting in positive and negative selection, it has been suggested that MHC molecules on selecting cells in the thymus are different from the MHC molecules in the peripheral tissues. Novel MHC molecules (142) and epitopes (143) have been observed in the medulla of the thymus. In a variation of the same theme, it has been suggested that the self-peptide bound to the MHC molecule in the thymus is novel. There is some evidence to suggest that thymic epithelial cells differ in processing and presentation of antigen (144). Consistent with this is the observation that radiation bone marrow chimeras (K^b x K^{bm3})F1 -> K^{bm3}, in which K^{bm3} differs from K^b only by a few residues in the antigen binding groove, were unable to positively select for T cells able to respond to ovalbumin and K^b

(145), in spite of the presence of K^b expressing APC in the periphery. However, it has also been shown that only medullary, not cortical, epithelial cells can act as antigen presenting cells (146), unless interleukin-1β is added to the cell culture (147). While there is extensive evidence for positive and negative selection of the T cell repertoire, little is known about the mechanisms by which these processes occur.

Tolerance

Tolerance is the absence of reactivity or immune response to self molecules by both T cells and B cells. In the case of T cells, the TCR can recognize antigen in association with self MHC molecules, but under normal physiological conditions in the absence of antigen, there is no recognition of the self MHC molecules by the T cells. Tolerance also extends, for the most part, to self molecules other than MHC. Tolerance is established in part during negative selection, however, extrathymic induction of tolerance has been demonstrated with transgenic mice which express class I (148) or class II (149) MHC molecules on a variety of tissues other than the thymus. All of these transgenic mice are tolerant in that no lymphocytic infiltration can be detected in any tissue examined. Usually the transgenic mice are tolerant as measured by *in vitro* assays or acceptance of skin grafts (148, 149). Tolerance can be

established by clonal deletion, anergy, or suppression of the autoreactive cell.

Extrathymic deletion

The deletion of immature thymocytes has been discussed in the previous section, and clonal deletion of B cells (150) is beyond the scope of this thesis. Clonal deletion of T cells may also occur in the periphery. Mls-1^a mice, treated from birth with anti-class II MAb to block deletion of V$\beta6^+$ T cells, gradually loose V$\beta6^+$ T cells from the periphery once the antibody treatment is terminated and the thymus removed (151). Injection of Mls-1^a spleen cells into thymectomized Mls-1^b recipients causes an initial expansion of V$\beta6^+$ T cells in the lymph nodes, followed by a reduction in frequency of V$\beta6^+$ T cells (152). Similarly, cells from mice transgenic for H-Y antigen and D^b injected into male nude recipients, initially expand in the periphery, but by 2 months post-injection, are greatly reduced in frequency. When the remaining transgenic TCR^+ T cells are examined, they have reduced levels of TCR and CD8 (153). These cells appear similar to the $CD8^{lo}$ T cells bearing the transgenic TCR (specific for H-Y and D^b) present in the periphery of males (154). It appears that when mature T cells encounter superantigen in the periphery, the initial proliferative response is followed by deletion and anergy, induced by down-regulation of TCR on the remaining T cells.

Anergy

Anergy is the non-responsiveness of T cells; the cells express TCR capable of recognizing the self molecule but lack the capacity to be activated. Both extrathymic and thymic induction of anergy has been demonstrated in many different systems of tolerance induction in B cells (150) and T cells, but I will review only the work concerning T cell tolerance. Intravenous injection of Mls-1^a spleen cells into Mls-1^b recipients may not induce clonal deletion, but the V$\beta6^+$ T cells in such treated mice cannot proliferate in response to Mls-1^a stimulating cells or to exogenous IL-2 (155, 156). In other systems, neonatally thymectomized Mls-1^a mice and Mls-1^b -> Mls-1^a chimeras have V$\beta6^+$ T cells which do not proliferate in response to Mls-1^a stimulating cells or anti-TCR MAb (133, 135). Also, Mls-1^a mice transgenic for a TCR β chain with Vβ8.1, which imparts reactivity to Mls-1^a, had a large frequency of $CD4^+$V$\beta8.1^+$ T cells in the periphery which were unresponsive to anti-CD3 MAb as well as Mls-1^a and SEB (157). Similarly to Mls-1^a expressing cells, enterotoxins can induce tolerance when high doses are injected intravenously. One week after injection, $CD4^+$V$\beta8^+$ T cells are present at normal frequency among splenocytes, but are unable to proliferate in response to SEB or exogenous interleukin-2 (158, 159). In this case, anergy may be induced by the binding of SEB to the TCR in the absence of class II molecules (96); this is consistent with the two-signal hypothesis of cell activation.

The two-signal hypothesis states that cells require two signals for full activation, and receiving only one signal results in paralysis (160). Since its original conception, the theory has been extended to specifically include T cells and cytokines (161). There is some indirect evidence from *in vitro* experiments to support the theory. Anergy can be induced in T cell clones by exposing them to (1) antigen presented by either (a) APC, which have been treated by chemical fixation, or (b) a planar membrane system which includes MHC molecules, or (2) anti-CD3 Ab immobilized on the culture dish (162a), or (3) a T cell mitogen, concanavalin A, in the absence of APC (161). In all of these cases, T cell lines or clones could not be stimulated subsequently by antigen and normal APC. It is presumed that each of the above culture conditions cannot provide both signals to the reactive T cells, because cytokine or some cell surface molecule is lacking. Although this mechanism may have a role to play in some forms of tolerance, it cannot be the only mechanism since this hypothesis cannot explain the initial transitory activation which has been observed to precede anergy in some systems (78).

Suppression

Although clonal deletion and anergy can account for tolerance in the systems described above, these theories cannot explain observations made in other experimental systems. For example, neonatal thymectomy leads to a variety of autoimmune disorders, however the development of the disorders can be prevented by

injection of $CD4^+$ spleen cells from normal syngeneic animals (162). Also, H-2^b mice do not respond to pork insulin, as measured by antibody production, but elimination of $CD8^+$ T cells from responding mice before immunization leads to the production of anti-pork insulin antibodies (163). Similarly, depleting $CD8^+$ T cells from the responding population of splenocytes increases their proliferation in a syngeneic MLR (in which irradiated, stimulating cells are genetically identical to the responding cells, 164). There are also several other examples of pre-immunization with antigen, either by a different route or in an altered form, which prevents the usual response to the antigen, measured as antibody production or cytolytic killing of target cells (165, 166). These results imply that suppression is acting on cells which would otherwise respond to the antigen. Suppression can be demonstrated by the transfer of unresponsiveness to either naive animals or *in vitro* assays. Splenocytes from tolerized animals injected into naive animals can render the recipients specifically tolerant to the original antigen (167, 168)

Many other studies (which will not be discussed here) have examined the specificity and restrictions of the suppressor cells involved, the results of which have led to the proposal of a complex cascade of suppressor inducer, suppressor transducer, suppressor effector T cells and soluble suppressor factors, with differing restrictions and specificities, required to generate suppression (169). Recently, a simpler model has been proposed based on

functional subsets of $CD4^+$ and $CD8^+$ T cells (170). The distinguishing features of this model are that $CD8^+$ T cells can be classified into Type 1 and Type 2 cells based on their pattern of lymphokines secreted, that suppressor cells differ from the other functional subsets of T cells by having specificities for idiotypes of other TCR, and that suppressor factors may be shed TCR molecules and secreted lymphokines. Common to both models is the anti-idiotypic specificity of suppressor cells; there are many examples of suppressor cells with anti-idiotypic specificities (171 - 173). For example, autoreactive T cells, activated in a syngeneic MLR, can induce suppression in recipient rats and mice when injected into the hind footpads. Splenocytes from the recipients do not proliferate in a syngeneic MLR, and inhibit the proliferation of splenocytes from naive animals in syngeneic MLR (174). Also, $CD8^+$ suppressor T cells can be generated by co-culture with $CD4^+$ T clones specific for allogeneic MHC molecules. These suppressor cells proliferate in response to the $CD4^+$ T cells not the allogeneic MHC molecules (175). Similarly, a suppressor T cell line, isolated from mice made tolerant to bovine serum albumin (BSA), inhibits proliferation of T cell lines in response to BSA by lysing the BSA specific T cells (176). $CD8^+$ suppressor T cells can be isolated from rats immunized with a $CD4^+$ T cell line specific for myelin basic protein (MBP), which can induce experimental autoimmune encephalomyelitis (EAE). The $CD8^+$ suppressor cells proliferate in response to and specifically lyse the MBP specific T cell line, not other $CD4^+$ T cells of different specificities, and can inhibit the

induction of EAE by co-injection with the MBP specific T cells (177). A suppressor T cell clone generated in a similar manner, blocks the proliferation of another MBP specific T cell clone in response to MBP (178). In an extension of these studies, rats were immunized, not with T cells, but with a peptide, the sequence of which corresponds to the CDR2 region of the TCR expressed by the MBP specific T cells. These immunized rats are protected from induction of EAE (179 - 181). The T cells specific for the TCR-peptide proliferate in response to the TCR-peptide as well as to the MBP specific T cell line. The TCR-peptide specific T cells do not exhibit suppressive activity *in vitro*, but can inhibit the induction of EAE by injection of MBP (181).

Thus, it appears that antigen-specific T cells can themselves be recognized by suppressor cells. It has been suggested that presentation of peptide fragments of the TCR by T cells to suppressor cells may lead to down-regulation of the presenting T cells (182). Immunizing rats with a TCR-peptide to generate T cells able to proliferate in response to other T cells bearing that TCR and inhibit *in vivo* function, supports this idea (181). There is also direct evidence that activated human T cells (which bear class II and class I MHC molecules) can present antigen to class II restricted T cells (183). It is an obvious extension for T cells to present endogenously synthesized TCR on class I MHC molecules. Suppression, then, can be demonstrated in many systems of

tolerance induction, and in some cases, the TCR may be the specific antigen recognized by the suppressor T cells.

Autoimmunity

Recognition of self peptides

In spite of the many mechanisms to ensure tolerance to self molecules, recognition of self and destruction of tissues may still occur. Autoimmunity, then, results in the absence of tolerance. MHC molecules do not distinguish between self and foreign peptides. Self peptides can compete for binding to class I and class II MHC molecules with foreign peptides. This has been demonstrated for peptides of murine cytochrome c (184), murine lysozyme (185, 186), rat MBP (187), murine class I and class II molecules (188, 189). These self-peptide-MHC complexes are functional, in that they can stimulate antigen-specific T cells. APC isolated from many different murine tissues can stimulate syngeneic T cell hybridomas, specific for murine hemoglobin (Hb), in the absence of exogenous Hb, implying that these APC process and present peptide fragments of Hb (190). B lymphoma cells can present peptides of their own immunoglobulins, processed through the endocytic pathway, on class II molecules (191, 192). Schwann cells, induced to express class II molecules by treatment with interferon-gamma (IFNγ), can present MBP to a syngeneic T cell clone specific for MBP (193). There also exist peripheral T cells

which can respond to peptides of self molecules, such as class I and class II molecules (188, 189), hemoglobin (194), and TCR molecules (Chapter 3, 181, 195). These autoreactive T cells are induced by immunization with the peptide fragment of the self molecule, or by *in vitro* culture with a peptide of a heterologous allele of the molecule. These potentially autoreactive T cells may evade tolerance induction because some minor antigenic determinants of self molecules are available in low amounts after *in vivo* processing and presentation (196, 197). This is demonstrated by the finding that CTL lines established by *in vitro* culture with APC and exogenous peptides of β_2-microglobulin or Hb cannot lyse target tumour cells unless the exogenous peptide is incubated with the target cells (194). The only omission of this study was a test of APC isolated from tissues of syngeneic mice for lysis by the autoreactive CTL lines.

Since cells can present self peptides on class I and class II MHC molecules, and autoreactive T cells are present in the periphery, the question then becomes: What induces autoimmunity? It may be that an initial viral, bacterial, or parasitic infection can either (a) cause tissue damage resulting in release of self antigens normally not exposed to the immune system, and production of inflammatory lymphokines, which induce expression of class II molecules by cells normally class II-negative, or (b) induce activation of autoreactive T cells through cross-reactivity of TCRs for the pathogenic antigen and a self epitope (198). The latter possibility, termed molecular

mimicry, has some support from a study in which experimental autoimmune uveitis (EAU) was induced in rats by immunization with peptides of viral proteins. The T cells specific for viral peptides also showed some reactivity to the autoantigen, S-Antigen, which is the target antigen of EAU (199). Unfortunately induction of EAU by infection with the viruses was not tested. Thus the balance of the immune system between tolerance to self and to foreign molecules may be upset by infection or deliberate (experimental) priming leading to autoreactivity.

NOD model of type-1 diabetes

The nonobese diabetic (NOD) strain was originally developed by selective inbreeding of the ICR strain. NOD mice spontaneously develop an autoimmune disorder which has many of the characteristics of type-1 diabetes, including lymphocytic infiltration of pancreatic islets of Langerhans, specific destruction of islet β cells, and severe hyperglycemia (200). The incidence of spontaneous diabetes varies among the different colonies of NOD mice, ranging between 70 to 95 percent among females by 250 days of age. The incidence among males is lower, ranging between 10 to 50 percent. This variation implies that some environmental factor may contribute to the induction of diabetes; diet and viral infection have been implicated in the induction of diabetes in the BB rat (201) and other mouse strains (202), respectively. Three recessive genes contribute to development of diabetes in NOD mice, one of which is linked to the MHC (203, 204). The MHC itself may

contribute to NOD susceptibility to diabetes. The H-2 haplotype of NOD is K^d and D^b for class I loci, and a unique I-A^{nod} for class II locus; I-E is not expressed in NOD mice due to a non-functional Eα gene (205). The β chain of I-A^{nod} has a unique β1 domain with several substitutions, including two amino acids which are normally conserved among the I-A alleles (206). One of these substitutions, the substitution of aspartic acid at position 57, is shared with a human HLA-DQβ allele also associated with susceptibility to diabetes (207). Position 57 is located on the α-helix of the antigen binding groove, so that it may be important in binding the autoantigen for presentation to, or the selection of, autoreactive T cells.

The effector cells of diabetes in NOD are bone marrow derived (208, 209). Macrophages have been implicated by cytotoxicity of macrophages against β cells *in vitro* (210), however, there is a large body of evidence implicating T cells. Neonatal thymectomy in NOD mice reduces the incidence of diabetes (211). Also, the infiltrating lymphocytes in the pancreas are mostly $CD4^+$ T cells, and continuous administration of anti-$CD4^+$ MAb can prevent diabetes in NOD mice (212). Similarly, depletion of $CD4^+$ T cells from diabetic NOD mice coupled with islet transplantation reverses hyperglycemia; when anti-CD4 MAb treatment is terminated, the replacement of peripheral $CD4^+$ T cells correlates with recurrence of hyperglycemia (213). In addition, diabetes can be transferred to young, irradiated, naive NOD mice by intravenous injection of $CD4^+$

and CD8+ splenocytes from diabetic NOD mice (214). T cell clones isolated from the pancreas of diabetic NOD mice, can also transfer diabetes and proliferate *in vitro* in response to NOD islet cells (215).

Other T cell-mediated autoimmune diseases exhibit a bias in the usage of TCR-Vβ by effector cells, such as rheumatoid arthritis (216, 217), and perhaps multiple sclerosis (218, 219). The repertoire of T cells in diabetes in NOD has been extensively investigated for this phenomenon. Islet-reactive T cells or T cells isolated from islets express a variety of Vβ and Vα (220 - 222). Also, NOD which lack nearly one third of genomic Vβ gene segments (223) or are transgenic for TCR Vβ8.2 still develop diabetes (224), indicating that a restricted TCR repertoire does not abrogate induction of diabetes. However, Vβ3+ T cells have been observed in islet infiltrates (216, 220, 225), in spite of the deletion of Vβ3+ T cells due to the expression of mtv-3 in NOD mice (76). The increased expression of Vβ3 among infiltrating T cells early in insulitis compared to peripheral T cells may indicate a role for Vβ3+ T cells in the pathogenesis of diabetes in NOD mice (225). Thus type-1 diabetes in NOD is an autoimmune disease involving the expression of an aberrant class II molecule, and heterogeneous TCR usage by infiltrating T cells.

Prevention of diabetes is possible through various forms of immunotherapy, including injection of antibodies to class II molecules (226) or CD4 (212), immunization with hsp-65 (227),

bone marrow transplantation, and administration of cyclosporin A (200). Recently, it has been demonstrated that a single immunization with complete Freund's adjuvant (CFA) at an early age can protect NOD mice from diabetes (228). Also, protection can be transferred with T cells from immunized mice (229). Conversely, protection from diabetes by injection of live *Bacillus* Calmett-Guerin (BCG) can be transferred with macrophages but not T cells from immunized NOD mice (230). Thus regulation of diabetes is a complex affair involving many regulatory cell types.

Experimental allergic encephalomyelitis

Experimental allergic encephalomyelitis (EAE) has many characteristics in common with MS (231), which make EAE a potential model for this putative autoimmune disease. EAE is characterized by lymphocytic infiltration into perivascular areas of the central nervous system (CNS), causing demyelinization and paralysis. Susceptibility to induction of EAE, by immunization with myelin basic protein (MBP), is linked to the MHC (232), and autoreactive T cells, specific for MBP, mediate EAE. Inflammatory lesions in the perivascular areas of the CNS contain B cells, T cells, as well as class II bearing cells, however, during the active chronic stage of EAE, T cells appear to be the most numerous cell type present (233). Neonatal thymectomy of rats abrogate the induction of EAE, and reconstitution with syngeneic thymocytes renders the recipients susceptible to EAE once again (234). Also, anti-CD4 MAb treatment can reverse the progression of EAE (235), and EAE can be

transferred to unimmunized animals with $CD4^+$ T cells from animals with EAE (236). T cell clones specific for MBP can also induce EAE without immunization with MBP (237).

The TCR repertoire of T cells reactive to MBP is very restricted. In mice strains of $H\text{-}2^u$, 80% of MBP- specific T cells use Vβ8, 60% use Vα2, and 40% use Vα4 (238, 239); in Lewis rat, all MBP-specific T cells use Vβ8, and 70% use Vα2 (239, 240). The homogeneity in the T cell response to MBP has been exploited in methods of immunotherapy employed to treat EAE. Injection of anti-Vβ8 MAb can abrogate the induction of EAE and enhance the recovery from EAE (241, 242). As previously mentioned, immunization with attenuated T cell clones specific for MBP (177, 178), or with peptides of the TCR-Vβ8 sequence (179 - 181) can protect recipients from subsequent induction of EAE. However, other studies using the same peptides do not duplicate these results (243, 244), indicating this method of immunotherapy may not be reliable. The induction of anti-TCR T cells observed in the former group of studies may depend on some environmental factor or immunological history of the animal populations, which may influence the heterogeneity of the TCR repertoire and alter anti-idiotypic networks of experimental animals. In summary, EAE is a T cell mediated autoimmune disease in which homogeneity of Vβ usage by autoreactive T cells allows for novel immunotherapies to be employed to regulate the immune system.

Regulation of T cell responses

T cells can be subdivided into at least two distinct subsets based on the pattern of lymphokine secretion. Type 1 $CD4^+$ T cells (Th1) secrete interleukin 2 (IL-2), IL-3, interferon-gamma (IFNγ), tumor necrosis factor (TNF), transforming growth factor-beta (TGFβ), and granulocyte-macrophage-colony stimulating factor (GM-CSF), whereas Type 2 $CD4^+$ T cells (Th2) secrete IL-3, IL-4, IL-5, IL-6, IL-10, TNF, and GM-CSF (245). There is also some evidence for a third Th subset, Th0, secreting IL-4, IL-5, IL-10, IFNγ, and IL-2, which may be a precursor to or an intermediate between Th1 and Th2 cells (246). This protocol of subdivision may extend to T cells of the $CD8^+$ phenotype. Type 1 $CD8^+$ T cells secrete IFN-γ, IL-6, IL-2, and TNF; Type 2 $CD8^+$ T cells secrete IL-2, IL-4, IL-5, IFN-g, TNF, and GM-CSF. (247). The subsets of T cells appear to correlate with function. Th1 and Th2 cells can enhance the generation of CTL and B cell responses, but Th2 cells induce proliferation of B cells and secretion of IgG, IgA, and IgE (245), whereas Th1 cells can be cytotoxic to B cells and other APC and do not induce production of IgE (248). Delayed type hypersensitivity (DTH) is mediated by Th1 only (245). Type 1 $CD8^+$ T cells are cytotoxic T lymphocytes (CTL), and Type 2 $CD8^+$ T cells can suppress the proliferation of antigen specific T cells (247).

Th1 and Th2 cells can each regulate the function of the other through the cytokines they secrete (249). IFNγ, produced by Th1

cells, inhibits the proliferation of Th2 cells, and inhibits many functions of IL-4, produced by Th2 cells. Also, IFNγ promotes the production of Th1 clones *in vitro* culture. IL-10, produced by Th2 cells, inhibits the secretion of cytokines by and proliferation of Th1 cells. In addition, IL-4 can inhibit the production of IFNγ by mononuclear cells and promotes the production of Th2 clones *in vitro* culture. Thus Th1 and Th2 cells can be viewed as suppressor cells; each type suppressing the proliferation and function of the other (170). In this light, the induction of suppression of B cell response to HEL by different epitopes (250) can be interpreted as the preferential induction of Th1 cells over Th2 cells. There is some evidence that Th1 and Th2 cells are induced by different antigens. For example, T cell clones specific for chicken red blood cells are usually Th1 cells, whereas T cell clones specific for fowl γ-globulin or keyhole lympet hemocyanin are usually Th2 cells (251). Also within the T cell response to *Leishmania major*, T cell clones which exacerbate leishmaniasis are Type 1, and T cell clones which protect against the disease are Type 2. Exacerbation of and protection from leishmaniasis can be induced by different parasitic antigens (252, 253). Consistent with this is the finding that antibody responses and DTH responses can be induced by different peptide epitopes of the same protein (256). In addition, the same antigen may induce different T cell subsets in mice of different MHC haplotypes, as demonstrated with *L. major* (245), and type IV collagen (255). Thus regulation of T cell responses may be achieved by one subset of T cells secreting cytokines which inhibit the function and

proliferation of another subset. This type of regulation differs from suppression of T cell responses (as discussed in a previous section), which acts through lysis or negative signalling of potentially responsive T cells, but may appear as suppression of DTH or B cell responses.

Project and rational

Regulation includes selection of an antigen-reactive and self-tolerant repertoire of lymphocytes, interacting lymphokines, and perhaps an immune network of the immune system. The idiotypic network theory proposes that there are lymphocytes which recognize epitopes of antigen receptors (idiotopes) and these lymphocytes can influence the function of other lypmphocytes bearing these idiotopes (256). There is some evidence that antibodies can recognize V regions of other antibodies (257), and injection of these anti-idiotypic antibodies can affect the levels of the idiotypic antibodies in sera (258), indicating some interaction among the antibodies. Originally proposed for a network among antibodies, this theory can be extended to T cells and their particular form of antigen recognition (170, 259). The evidence for anti-idiotypic T cells has been discussed in a previous section. In addition, 10% of splenic T cells from specific pathogen-free mice are large blast cells, which can be shown to enhance or suppress the production of antibodies. These "natural effector" T cells are

interpreted as a component of the immune network (260). If T lymphocytes can recognize peptides derived from the variable region of antibodies and TCRs and influence the response of lymphocytes which express those receptors, then one may hypothesize that these peptides are regulatory elements involved in the control of immune responses.

Although there has been some speculation on the regulatory role of peptides of the variable region of the TCR in immune responses, much of the work has been limited to conflicting studies on the effect of a Vβ8.2-peptide on the induction of EAE in rats (179 - 181, 243, 244). We therefore had to clarify the role of Vβ-peptides in regulation by investigating their effect on other T cell responses. Our first objective was to study the *in vitro* effect of Vβ-peptides on T cell responses. Specifically we studied the effect of Vβ6-peptides on the response of T cell hybridomas, which express Vβ6, to superantigen Mls-1^a. Most T cells expressing Vβ6 respond to Mls-1^a, however the nature of interaction between Mls and TCR had not been elucidated. Unlike peptide antigens, recognition of Mls is not restricted to the MHC haplotype of the responding T cell, does not require co-signalling through CD4 and CD8, and involves primarily the β chain of the TCR. Therefore, we tested several peptides of partially overlapping sequences from amino acid position 1 to 75 of the Vβ6 gene segment on the *in vitro* stimulation of $V\beta6^+$ T cell hybridomas with splenocytes from Mls-1^a mice. The *in vitro* effect of these peptides on the response of the T cell hybridomas enabled

us to identify the putative binding site on the β chain of the TCR which interacts with Mls-1^a (Chapter II).

Another prediction of the network theory is that the immune system is not tolerant to the variable regions of the Ab or TCR. This has not been explored for T cell responses to the variable region of the TCR. Our second objective was therefore to study the immunogenicity of peptides of TCR-Vβ gene segments. We tested the immunogenicity of several Vβ6-peptides and peptides of the N-terminal of Vβ3, Vβ8.1, Vβ8.2, and Vβ8.3 in several strains of mice: BALB/c (H-2^d, Mls-1^b2^a), DBA/2J (H-2^d, Mls-1^a2^a), CBA/CaJ (H-2^d, Mls-1^b2^b), CBA/J (H-2^k, Mls-1^a2^a), C57Bl/6J (H-2^b, Mls-1^b2^b), and D1.LP (H-2^b, Mls-1^a2^a). We compared the immunogenicity of the TCR-peptides between strains of different MHC haplotypes and between strains of different Mls phenotypes to determine if there was an Ir effect in responsiveness or if the deletion of the Vβ6, Vβ8, or Vβ3 TCR influenced tolerance to these peptides. Our reasoning was that the presence of T cells bearing a particular Vβ may induce tolerance to peptides of that Vβ and that nonimmunogenic peptides could not exert an influence on immune responses (Chapter III).

Our final objective was to study the effects of TCR-peptides *in vivo*. We pre-immunized mice with each of the Vβ6-peptides and then studied the response of lymphocytes to either Mls-1^a or a peptide antigen $(EYA)_5$. As stated before Mls-1^a stimulates $V\beta6^+$ T cells,

and in BALB/c mice, 50% of T cells which respond to $(EYA)_5$ are $V\beta6^+$ (261). Thus we could investigate the effect of Vβ6-peptides *in vivo* on the response of T cells bearing $V\beta6^+$ TCR, in two different antigen systems. In addition, we extended the study to a model of autoimmune disease. Since the pathogenesis of diabetes in NOD mice may involve $V\beta3^+$ T cells, we tested the effect of peptides from the TCR Vβ3 gene segment on the transfer of Type 1 diabetes in NOD mice. Diabetes can be transferred to young, naive NOD mice by intravenous injection of splenocytes from diabetic NOD mice. By co-injecting diabetogenic splenocytes and splenocytes from NOD which had been immunized with Vβ3-peptides, we were able to determine the effect of the Vβ3-peptides *in vivo* on the induction of autoimmunity (Chapter IV).

Bibliography

1. Kappler, J., R. Kubo, K. Haskins, J. White, and P. Marrack. 1983. The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. Cell 34:727-737.

2. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. J. Exp. Med. 157:1149-1169.

3. Weiss, A., and J.D. Stobo. 1984. Requirement for the coexpression of T3 and the T cell antigen receptor on a malignant human T cell line. J. Exp. Med. 160:1284-1299.

4. Brenner, M.B., J. McLean, D.P. Dialynas, J.L. Strominger, J.A. Smith, F.L. Owen, J.G. Seidman, S. Ip, F. Rosen, and M.S. Krangel. 1986. Identification of a putative second T-cell receptor. Nature 322:145-149.

5. Dembic, Z., W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer, and M. Steinmetz. 1986. Transfer of specificity by murine α and β T-cell receptor genes. Nature 320:232-238.

6. Kappler, J. R., Kubo, K. Haskins, C. Hannum, P. Marrack, M. Pigeon, B. McIntyre, J. Allison, and I. Trowbridge. 1983. The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. Cell 35:295-302.

7. Chien, Y.-h., D.M. Becker, T. Lindsten, M. Okamura, D.I. Cohen, and M.M. Davis. 1984. A third type of murine T-cell receptor gene. Nature 312:31-35.

8. Saito, H., D.M. Kranz, J. Takagaki, A.C. Hayday, H.N. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature 309:757-762.

9. Band, H. F. Hochstenbach, J. McLean, S. Hata, M.S. Krangel, M.B. Brenner. 1987. Immunochemical proof that a novel rearranging gene encodes the T cell receptor δ subunit. Science 238:682-684.

10. Manolios, N., F. Letourneur, J.S. Bonifacino, and R.D. Klausner. 1991. Pairwise, cooperative and inhibitory interactions describe the assembly and probable structure of the T-cell antigen receptor. EMBO J. 10:1643-1651.

11. Baniyash, M. P. Garcia-Morales, J.S. Bonifacino, L.E. Samelson, and R.D. Klausner. 1988. Disulfide linkage of the ζ and η chains of the T cell receptor. J. Biol. Chem. 263:9874-9878.

12. Samelson, L.E., M.D. Patel, A.M. Weissman, J.B. Harford, and R.D. Klausner. 1986. Antigen activation of murine T cells induces tyrosine phosphorylation of a polypeptide associated with the T cell antigen receptor. Cell 46:1083-1090.

13. Klein, J., F. Figueroa, and Z.A. Nagy. 1983. Genetics of the major histocompatibility complex: the final act. Ann. Rev. Immunol. 1:119-142.

14. Babbitt, B., P.M. Allen, G. Matsueda, E. Haber, and E. Unanue.1985. The binding of immunogenic peptides to Ia histocompatibility molecules. Nature 317:359-361.

15. Katz, D.K., T. Hamaoka, M.E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. The H-2 gene complex determines successfull physiologic lymphocyte interactions. Proc. Natl. Acad. Sci. USA 70:2624-2628.

16. Klein, J. 1979. The major histocompatibility complex of the mouse. Science 203:516-521.

17. Steinmetz, M., and L. Hood. 1983. Genes of the major histocompatibility complex in mouse and man. Science 222:727-733.

18. Cho, S., M. Attaya, and J.J. Monaco. 1991. New class II-like genes in the murine MHC. Nature 353:573-575.

19. Kelly, A.P., J.J. Monaco, S. Cho, and J. Trowsdale. 1991.. A new human HLA class II-related locus, DM. Nature 353:571-573.

20. Kappler, J.W., and P. Marrack. 1979. The role of H-2 linked genes in helper T-cell function IV. Importance of T-cell genotype and host environment in I-region and Ir gene expression. J. Exp. Med. 149:1510-1522.

21. Salter, R.D., R.J. Benjamine, P.K. Wesley, S.E. Buxton, T.P.J. Garrett, C. Clayberger, A.M. Krensky, A.M. Norment, D.R. Littman, and P. Parham. 1990. A binding site for the T-cell co-receptor CD8 on the α_3 domain of HLA-A2. Nature 345:41-46.

22. Konig, R., L.-Y. Huang, and R.N. Germain. 1992. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. Nature 356:796-798.

23. Veillette, A., M.A. Bookman, E.M. Horak, and J.B. Bolen. 1988. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tryrosine-protein kinase $p56^{lck}$. Cell 55:301-308.

24. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibilitiy antigen, HLA-A2. Nature 329:506-512.

25. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 329:512-518.

26. Ziegler, H.K., and E.R. Unanue. 1982. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. Proc. Natl. Acad. Sci. USA 79:175-178.

27. Shimonkevitz, R., J. Kappler, P. Marrack, and H. Grey. 1983. Antigen recognition by H-2-restricted T cells I. Cell-free antigen processing. J. Exp. Med. 158:303-315.

28. Morrison, L.M., V.L. Braciale, and T.J. Braciale. 1988. Antigen form influences induction and frequency of influenza-specific class I and class II MHC-restricted cytolytic T lymphocytes. J. Immunol. 141:363-368.

29. Sweeteser, M.T., L.A. Morrison, V.L. Braciale, and T.J. Braciale. 1989. Recognition of pre-processed endogenous antigen by class I but not class II MHC-restricted T cells. Nature 342:180-182.

30. Monaco, J.J., and H.O. McDevitt. 1981. Identification of a fourth class of proteins linked to the murine major histocompatibility complex. Proc. Natl. Acad. Sci. USA 79:3001-3005.

31. Goldberg, A.L., and K.L. Rock. 1992. Proteolysis, proteasomes and antigen presentation. Nature 357:375-379.

32. Brown, M.G., J. Driscoll, and J.J. Monaco. 1991. Structural and serological similarity of MHC-linked LMP and proteasome (multicatalytic proteinase) complexes. Nature 353:355-357.

33. Kelly, A., S.H. Powis, R. Glynne, E. Radley, S. Beck, and J. Trowsdale. Second proteasome-related gene in the human MHC class II region. Nature 353:667-668.

34. Parham, P. 1990. Transporters of delight. Nature 348:674-675.

35. Spies, T., V. Cerundolo, M. Colonna, P. Cresswell, A. Townsend, and R. DeMars. 1992. Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer. Nature 355:644-646.

36. Attaya, M. S. Jameson, C.K. Martinez, E. Hermel, C. Aldrich, J. Forman, K.F. Lindahl, M.J. Bevan, and J.J. Monaco. 1992. *HAM-2* corrects the class I antigen-processing defect in RMA-S cells. Nature 355:647-649.

37. Kleijmeer, M.J., A. Kelly, H.J. Geuze, J.W. Slot, A. Townsend, and J. Trowsdale. 1992. Location of MHC-encoded transporters in the endoplasmic reticulum and *cis*-Golgi. Nature 357:342-344.

38. Henderson, R.A., H. Michel, K. Sakaguchi, J. Shabanowitz, E. Appella, D.F. Hunt, and V.H. Engelhard. 1992. HLA-A2.1-associated peptides from a mutant cell line: a second pathway of antigen presentation. Science 255:1264-1266.

39. Wei, M.L., and P. Cresswell. 1992. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. Nature 356:443-446.

40. Hunt, D.F., R.A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A.L. Cox, E. Appella, and V.H. Engelhard. 1992. Characterization of peptides bound to class I MHC molecule HLA-A2.1 by mass spectrometry. Science 255:1261-1263.

41. Brodsky, F.M., and L.E. Guagliardi. 1991. The cell biology of antigen processing and presentation. Ann. Rev. Immunol. 9:707-744.

42. Nuchtern, J.G., J.S. Bonifacino, W.E. Biddison, and R.D. Klausner. 1989. Brefeldin A implicates egress from endoplasmic reticulum in class I restricted antigen presentation. Nature 339:223-226.

43. Sherman, L.A., T.A. Burke, and J.A. Biggs. 1992. Extracellular processing of peptide antigens that bind class I major histocompatibility molecules. J. Exp. Med. 175:1221-1226.

44. Elliott, T. 1991. How do peptides associate with MHC class I molecules? Immunol. Today 12:386-387.

45. Morrison, L.A., A.E. Lukacher, V.L. Braciale, D.P. Fan, and T.J. Braciale. 1986. Differences in antigen presentation to MHC class I-and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. J. Exp. Med. 163:903-921.

46. Guagliardi, L.E., B. Koppelman, J.S. Blum, M.S. Marks, P. Cresswell, and F.M. Brodsky. 1990. Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. Nature 343:133-139.

47. Peters, P.J., J.J. Neefjes, V. Oorschot, H.L. Ploegh, and H.J. Geuze. 1991. Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. Nature 1991. 349:669-679.

48. St.-Pierre, Y., and T.H. Watts. 1990. MHC class II-restricted presentation of native protein antigens by B cells is inhibitable by cycloheximide and brefeldin A. J. Immunol. 145:812-818.

49. Davidson, H.W., P.A. Reid, A. Lanzavecchia, and C. Watts. 1991. Processed antigen binds to newly synthesized MHC class II molecules in antigen-specific B lymphocytes. Cell 67:105-116.

50. Malnati, M.S., M. Marti, T. LaVaute, D. Jaraquemada, W. Biddison, R. DeMars, and E.O. Long. 1992. Processing pathways for presentation of cytosolic antigen to MHC class II-restricted T cells. Nature 357:702-704.

51. Brodsky, F.M. 1991. Intracellular routes for antigen processing and presentation. Institut Pasteur Res Immunol. 142:453-458.

52. Singh, B., K.-C. Lee, E. Fraga, A. Wilkinson, M. Wong, and M.A. Barton. 1980. Minimum peptide sequences necessary for priming and triggering of humoral and cell-mediated immune responses in mice: use of synthetic peptide antigens of defined structure. J. Immunol. 124:1336-1342.

53. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell 44:959-968.

54. Schwartz, R.H., B.S. Fox, E. Fraga, C. Chen, and B. Singh. 1985. The T lymphocyte response to cytochrome c V.

Determination of the minimal peptide size required for stimulation of T cell clones and assessment of the contribution of each residue beyond this size to antigenic potency. J. Immunol. 135:2598-2608.

55. Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature 348:252-254.

56. Jardetzky, T.S., W.S. Lane, R.A. Robinson, D.R. Madden, and D.C. Wiley. 1991. Identification of self peptides bound to purified HLA-B27. Nature 353:326-329.

57. Rudensky, A.Y., P. Preston-Hurlburt, S.-C. Hong, A. Barlow, and C.A. Janeway, Jr. 1991. Sequence analysis of peptides bound to MHC class II molecules. Nature 353:622-627.

58. Rotzchke, O., and K. Falk. 1991. Naturally occurring peptide antigens derived from the MHC class I-restricted processing pathway. Immunol. Today 12:447-455.

59. Allen, P.M. 1987. Antigen processing at the molecular level. Immunol. Today 8:270-273.

60. Boyer, M., Z. Novak, A. Fotedar, and B. Singh. 1988. Contribution of antigen processing to the recognition of a synthetic peptide antigen by specific T cell hybridomas. J. Mol. Recog. 1:99-106.

61. Sette, A., S. Buus, S. Colon, J.A. Smith, C. Miles, and H.M. Grey. 1987. Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. Nature 328:395-399.

62. Madden, D.R., J.C. Gorga, J.L. Strominger, and D.C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. Nature 353:321-329.

63. Schwartz, R.H. 1985. T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Ann. Rev. Immunol. 3:237-261.

64. Claverie, J.-M., A. Prochnicka-Chalufour, and L. Bougueleret. 1989. Implications of a Fab-like structure for the T-cell receptor. Immunol. Today 10:10-14.

65. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. Nature 334:395-401.

66. Jorgensen, J.L., U. Esser, B.F. de St. Groth, P.A. Reay, and M.M. Davis. 1992. Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics. Nature 355:224-230.

67. Nalefski, E.A., S. Kasibhatla, and A. Rao. 1992. Functional analysis of the antigen binding site on the T cell receptor α chain. J. Exp. Med. 175:1553-1563.

68. Danska,J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. J. Exp. Med. 172:27-33.

69. Matsui, K., J.J. Boniface, P.A. Reay, H. Schild, B.F. de St. Groth, and M.M. Davis. 1991. Low affinity interaction of peptide-MHC complexes with T cell receptors. Science 254:1788-1791.

70. Weber, S., A. Traunecker, F. Oliveri, W. Gerhard, and K. Karjalainen. 1992. Specific low-affinity recognition of major histocompatibility complex plus peptide by soluble T-cell receptor. Nature 356:793-796.

71. Williams, A.F., and A.D. Beyers. 1992. At grips with interactions. Nature 356:746-747.

72. Festenstein, H. 1974. Pertinent features of M locus determinants including revised nomenclature and strain distribution. Transplantation 18:555-557.

73. Abe, R, and R.J. Hodes. The Mls system: non-MHC genes that encode strong T-cell stimulatory determinants. Immunol. Today 9:230-235.

74. Herman, A., J.W. Kappler, P. Marrack, and A.M. Pullen. 1991. Superantigens: mechanism of T-cell stimulation and role in immune responses. Annu. Rev. Immunol. 9:74-772.

75. Woodland, D., M.P. Happ, J. Bill, and E. Palmer. 1990. Requirement for cotolerogenic gene products in the clonal deletion of I-E reactive T cells. Science 247:964-967.

76. Fairchild, S., A.M. Knight, P.J. Dyson, and K. Tomaonari. 1991. Co-segregation of a gene encoding a deletion ligand for Tcrβ-V3^{+} T cells with Mtv-3. Immunogenetics 34:227-230.

77. MacDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha-Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls^{a}-encoded antigens. Nature 332:40-45.

78. Abe, R., M.S. Vacchio, B. Fox, and R.J. Hodes. 1988. Preferential expression of the T-cell receptor Vβ3 gene by Mls^{c} reactive T cells. Nature 335:827-828.

79. Happ, M.P., D.L. Woodland, and E. Palmer. 1989. A third T-cell receptor β-chain variable region encodes reactivity to Mls-1^{a} gene products. Proc. Natl. Acad Sci. USA 86:6293-6296.

80. Kappler, J.W., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. A T cell receptor Vβ segment that imparts reactivity to a class II major histocompatibility complex product. Cell 49:263-271.

81. Vacchio, M.S., J.J. Ryan, and R.J. Hodes. 1990. Characterization of the ligand(s) responsible for negative selection of Vβ11- and Vβ12-expressing T cells: effects of a new Mls determinant. J. Exp. Med. 172:807-813.

82. Bill, J., O. Kanagawa, D.L. Woodland, and E. Palmer. 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of Vβ11-bearing T cells. J. Exp. Med. 169:1405-1419.

83. Johnson, H.M., J.K. Russell, and C.H. Pontzer. 1991. Staphylococcal enterotoxin microbial superantigens. FASEB J. 5:2706-2712.

84. Cole, B.C., D.R. Kartchner, and D.J. Wells. 1990. Stimulation of mouse lymphocytes by a mitogen derived from *Mycoplasma arthritidis* (MAM) VIII. Selective activation of T cells expressing distinct Vβ T cell receptors from various strains of mice by the "superantigen" MAM.

85. Marrack, P. and J. Kappler. 1990. The Staphylococcal enterotoxins and their relatives. Science 248:705-711.

86. Janeway, C. 1991. Immune recognition. Mls: makes a little sense. Nature 349:459-461.

87. Acha-Orbea, H., A.N. Shakhov, L. Scarpellino, E. Kolb, V. Muller, A. Vessaz-Shaw, R. Fuchs, K. Blochlinger, P. Rollini, J. Billotte, M. Sarafidou, H.R. MacDonald, and H. Diggelmann. 1991. Clonal deletion of Vβ14-bearing T cells in mice transgenic for mammary tumour virus. Nature 350:207-211.

88. Choi, Y., J.W. Kappler, and P. Marrack. 1991. A superantigen encoded in the open reading frame of the 3' long terminal repeat of mouse mammary tumour virus. Nature 350:203-207.

89. Mollick, J.A., R.G. Cook, and R.R. Rich. 1989. Class II MHC molecules are specific receptors for Staphylococcus enterotoxin A. Science 244:817-820.

90. MacDonald, H.R., R.K. Lees, and Y. Chvatchko. 1990. $CD8^+$ T cells respond clonally to Mls-1^a encoded determinants. J. Exp. Med. 171:1381-1386.

91. Chvatchko, Y., and H.R. MacDonald. 1991. $CD8^+$ T cell response to Mls-1^a determinants involves major histocompatibility complex class II molecules. J. Exp. Med. 173:779-782.

92. Sekaly, R.-P., G. Croteau, M. Bowman, P. Scholl, S. Burakoff, and R.S. Geha. 1991. The CD4 molecule is not always required for the T cell response to bacterial enterotoxins. J. Exp. Med. 173:367-371.

93. Janeway, C.A., Jr., J. Yagi, P.J. Conrad, M.E. Katz, B. Jones, S. Vroegop, and S. Buxser. 1989. T-cell responses to Mls and to bacterial proteins that mimic its behavior. Immunol. Rev. 107:61-87.

94. Kaye, J., and S.M. Hedrick. 1988. Analysis of specificity for antigen, Mls, and allogeneic MHC by transfer of T-cell receptor α- and β-chain genes. Nature 336:580-583.

95. Gascoigne, N.R.J., and K.T. Ames. 1991. Direct binding of secreted T-cell receptor b chain to superantigen associated with class II major histocompatibility complex protein. Proc. Natl. Acad. Sci. USA 88:613-616.

96. Hewitt, C.R.A., J.R. Lamb, J. Hayball, M. Hill, M.J. Owen, and R.E. O'Hehir. 1992. Major histocompatibility complex independent clonal T cell anergy by direct interaction of *Staphylococcus aureus* enterotoxin B with the T cell antigen receptor. J. Exp. Med. 175:1493-1499.

97. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T-cell-receptor β-chain that interact with *S. aureus* toxin superantigens. Nature 346:471-473.

98. Pullen, A.M., J. Bill, R.T. Kubo, P. Marrack, and J.W. Kappler. 1991. Analysis of the interaction site for the self superantigen Mls-1^a on T cell receptor Vβ. J. Exp. Med. 173:1183-1192.

99. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor β chain that interacts with the self-superantigen Mls-1^a. Cell 61:1365-1374.

100. MacNeil, D., E. Fraga, and B. Singh. 1992. Inhibition of superantigen recognition by peptides of the variable region of the T cell receptor β chain. Eur. J. Immunol. 22:937-941.

101. Dellabona, P., J. Peccoud, J. Kappler, P. Marrack, C. Benoist, and D. Mathis. 1990. Superantigens interact with MHC class II molecules outside the antigen groove. Cell 62:1115-1121.

102. Braunstein, N.S., D.A. Weber, X.-C. Wang, E.O. Long, and D. Karp. 1992. Sequences in both class II major histocompatibility complex α and β chains contribute to the binding of the superantigen toxic shock syndrome toxin 1. J. Exp. Med. 175:1301-1305.

103. Scollay, R. 1991. T-cell subset relationships in thymocyte development. Curr. Opinions Immunol. 3:204-209.

104. Strominger, J.L. 1989. Developmental biology of T cell receptors. Science 244:943-950.

105. Sprent, J. 1978. Restricted helper function of F1 -> Parent bone marrow chimeras controlled by K-end of H-2 complex. J. Exp. Med. 147:1838-1842.

106. Bevan, M.J. 1977. In a radiation chimaera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells. Nature 269:417-418.

107. Zinkernagel, R.M., G.N. Callahan, A. Althage, S. Cooper, P.A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? J. Exp. Med. 147:882-896.

108. Zinkernagel, R.M., G.N. Callahan, J. Klein, and G. Dennert. Cytotoxic T cells learn specificty for self H-2 during differentiation in the thymus. Nature 271:251-253.

109. Kruisbeek, A.M., J.J. Mond, B.J. Fowlkes, J.A. Carmen, S. Bridges, and D.L. Longo. 1985. Absence of the Lyt-2$^-$,L3T4$^+$ lineage of T cells in mice treated neonatally with anti-I-A correlates with absence of intrathymic I-A-bearing antigen-presenting cell function. J. Exp. Med. 161:1029-1047.

110. Kruisbeek, A.M., M.J. Fultz, S.O. Sharrow, A. Singer, and J.J. Mond. 1983. Early development of the T cell repertoire. *In vivo* treatment of neonatal mice with anti-I-A antibodies interferes with differentiation of I-restricted T cells but not K/D-restricted T cells. J. Exp. Med. 157:1932-1946.

111. Marusic-Galesic, S., D.A. Stephany, D.L. Longo, and A.M. Kruisbeek. 1988. Development of CD4$^-$CD8$^+$ cytotoxic T cells requires interactions with class I MHC determinants. Nature 333:180-183.

112. Zijlstra, M., M. Bix, N.E. Simister, J.M. Loring, D.H. Raulet, and R. Jaenisch. β2-microglobulin deficient mice lack CD4$^-$8$^+$ cytolytic T cells. Nature 34:742-746.

113. Grusby, M.J., R.S. Johnson, V.E. Papioannou, and L.H. Glimcher. 1991. Depletion of CD4$^+$ T cells in major histocompatibility complex class II-deficient mice. Science 253:1417-1420.

114. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Genoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. Cell 66:1051-1066.

115. Krimpenfort, P., F. Ossendorp, J. Borst, C. Melief, and A. Berns. 1989. T cell depletion in transgenic mice carrying a mutant gene for TCR-β. Nature 341:742-745.

116. MacDonald, H.R., R.K. Lees, R. Schneider, R.M. Zinkernagel, and H. Hengartner. 1988. Positive selection of CD4$^+$ thymocytes controlled by MHC class II gene products. Nature 336:471-473.

117. Blackman, M.A., P. Marrack, and J. Kappler. 1989. Influence of the major histocompatibility complex on positive thymic selection of Vβ17a$^+$ T cells. Science 244:214-217.

118. Kaye, J., M.-L. Hsu, M.-E. Sauron, S.C. Jameson, N.R.J. Gascoigne, and S.M. Hedrick. 1989. Selective development of CD4$^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. Nature 341:746-749.

119. Berg, L.J., A.M. Pullen, B.F. de St. Groth, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell 58:1035-1046.

120. Scott, B., H. Bluthmann, H.S. Teh, and H. von Boehmer. 1989. The generation of mature T cells requires interaction of the αβ T-cell receptor with major histocompatibility antigens. Nature 338:591-593.

121. Longo, D.L., A.M. Kruisbeek, M.L. Davis, and L.A. Matis. 1985. Bone marrow-derived thymic antigen-presenting cells determine self-recognition of Ia-restricted T lymphocytes. Proc. Natl. Acad. Sci. USA 82:5900-5904.

122. van Ewijk, W., Y. Ron, J. Monaco, J. Kappler, P. Marrack, M. LeMeur, P. Gerlinger, B. Durand, C. Benoist, and D. Mathis. 1988. Compartmentalization of MHC class II gene expression in transgenic mice. Cell 53:357-370.

123. Benoist, C., and D. Mathis. 1989. Positive selection of the T cell repertoire: where and when does it occur? Cell 58:1027-1033.

124. Egerton, M., R. Scollay, and K. Shortman. 1990. Kinetics of mature T-cell development in the thymus. Proc. Natl. Acad. Sci USA 87:2579-2582.

125. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature 332:35-40.

126. White, J., A. Herman, A.M. Pullern, R. Kubo, J.W. Kappler, and P. Marrack. 1989. The Vβ specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell 56:27-37.

127. Fowlkes, B.J., R.H. Schwartz, and D.M. Pardoll. 1988. Deletion of self-reactive thymocytes occurs at a $CD4^+8^+$ precursor stage. Nature 334:620-623.

128. MacDonald, H.R., H. Hengartner, and T. Pedrazzini. 1988. Intrathymic deletion of self-reactive cells prevented by neonatal anti-CD4 antibody treatment. Nature 335:174-176.

129. Kisielow, P., H. Bluthmann, U.D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. Nature 333:742-746.

130. von Boehmer, H. 1990. Developmental biology of T cells in T cell-receptor transgenic mice. Annu. Rev. Immunol. 8:531-556.

131. Dent, A.L., L.A. Matis, F. Hooshmand, S.M. Widacki, J.A. Bluestone, and S.M. Hedrick. 1990. Self-reactive γδ T cells are eliminated in the thymus. Nature 343:714-719.

132. Hengartner, H., B. Odermatt, R. Schneider, M. Schreyer, G. Walle, H.R. MacDonald, and R.M. Zinkernagel. 1988. Deletion of self-reactive T cells before entry into the thymus medulla. Nature 336:388-390.

133. Jones, L.A., L..T. Chin, G.R. Merriam, L.M. Nelson, and A.M. Kruisbeck. 1990. Failure of clonal deletion in neonatally thymectomized mice: tolerance is preserved through clonal anergy. J. Exp. Med. 172:1277-1285.

134. Marrack, P., D. Lo, R. Brinster, R. Palmiter, L. Burkly, R.H. Flavell, and J. Kappler. 1988. The effect of thymus environment on T cell development and tolerance. Cell 53:627-634.

135. Ransdell, F., T. Lantz, and B.J. Fowlkes. 1989. A nondeletional mechanism of thymic self tolerance. Science 246:1038-1041.

136. Bandeira, A., A. Coutinho, O. Burlen-Defranoux, I. Khazaal, M. Coltey, F. Jacquemart, N. LeDouarin, and J. Salaun. 1992. Thymic epithelium induces neither clonal deletion nor anergy to Mls 1^a antigens. Eur. J. Immunol. 22:1397-1404.

137. Smith, C.A., G.T. Williams, R. Kingston, E.J. Jenkinson, and J.J.T. Owen. 1989. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. Nature 337:181-184.

138. Matzinger, P., and S. Guerder. 1989. Does T-cell tolerance require a dedicated antigen-presenting cell? Nature 338:74-76.

139. Murphy, K.M., A.B. Heimberger, D.Y. Loh. 1990. Induction by antigen of intrathymic apoptosis of $CD4^+CD8^+$ TCR^{lo} thymocytes *in vivo*. Science 250:1720-1723.

140. Swat, W., L. Ignatowicz, H. von Boehmer, and P. Kisielow. 1991. Clonal deletion of immature $CD4^+8^+$ thymocytes in suspension culture by extrathymic antigen-presenting cells. Nature 351:150-153.

141. Sprent, J., D. Lo, E.-K. Gao, and Y. Ron. 1988. T cell selection in the thymus. Immunol Rev. 101:173-190.

142. Karisson, L., C.D. Surh, J. Sprent, and P.A. Peterson. 1991. A novel class II MHC molecule with unusual tissue distribution. Nature 351:485-488.

143. Murphy, D.B., D. Lo, S. Rath, R.L. Brinster, R.A. Flavell, A. Slanetz, and C.A. Janeway, Jr. 1989. A novel MHC class II epitope expressed in thymic medulla but not cortex. Nature 338:765-768.

144. Grant, E.P., and K.L. Rock. 1992. MHC class I-restricted presentation of exogenous antigen by thymic antigen-presenting cells *in vitro* and *in vivo*. J. Immunol. 148:13-18.

145. Nikolic-Zugic, J., and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. Nature 344:65-67.

146. Mizuochi, T., M. Kasai, T. Kokuho, T. Kakiuchi, and K. Hirokawa. 1992. Medullary but not cortical thymic epithelial cells present soluble antigens to helper T cells. J. Exp. Med. 175:1601-1605.

147. Lorenz, R.G., and P.M. Allen. 1989. Thymic cortical epithelial cells lack full capacity for antigen presentation. Nature 340:557-559.

148. Miller, J.F.A.P., G. Morahan, J. Allison, P.S. Bhathal, and K.O. Cox. 1989. T-cell tolerance in transgenic mice expressing major histocompatibility class I molecules in defined tissues. Immunol. Rev. 107:109-123.

149. Lo, D., L.C. Burkly, G. Widera, C. Cowing, R.A. Flavell, R.D. Palmiter, and R.L. Brinster. 1988. Diabetes and tolerance in transgenic class II MHC molecules in pancreatic beta cells. Cell 53:159-168.

150. Goodnow, C.C., S. Adelstein, and A. Basten. 1990. The need for central and peripheral tolerance in the B cell repertoire. Science 248:1373-1379.

151. Jones, L.A., T. Chin, D.L. Longo, and A.M. Kruisbeek. 1990. Peripheral clonal elimination of functional T cells. Science 250:1726-1729.

152. Webb, S., C. Morris, and J. Sprent. 1990. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell 63:1249-1256.

153. Rocha, B., and H. von Boehmer. 1991. Peripheral selection of the T cell repertoire. Science 251:1225-1228.

154. Teh, H.-S., H. Kishi, B. Scott, and H. von Boehmer. 1989. Deletion of autospecific T cells in T cell receptor (TCR)

transgenic mice spares cells with normal TCR levels and low levels of CD8 molecules. J. Exp. Med. 169:795-806.

155. Rammensee, H.-G., R. Kroschewski, and B. Frangoulis. 1989. Colonal anergy induced in mature Vβ6$^+$ T lymophocytes on immunizing Mls-1^b mice with Mls-1^a expressing cells. Nature 339:541-544.

156. Dannecker, G., S. Mecheri, L. Staiano-Coico, and M.K. Hoffmann. 1991. A characteristic Mls-1^a response precedes Mls-1^a anergy in vivo. J. Immunol. 146:2083-2087.

157. Blackman, M.A., H. Gerhard-Burgert, D.L. Woodland, E. Palmer, J.W. Kappler, and P. Marrack. 1990. A role for clonal inactivation in T cell tolerance to Mls-1^a. Nature 345:540-542.

158. Kawabe, Y., and A. Ochi. 1990. Selective anergy of Vβ8$^+$, CD4$^+$ T cells in staphylococcus enterotoxin B-primed mice. J. Exp. Med. 172:1065-1070.

159. Rellahan, B.L., L.A. Jones, A.M. Kruisbeek, A.M. Fry, and L.A. Matis. 1990. In vivo induction of anergy in pheripheral Vβ8$^+$ T cells by staphylococcal enterotoxin B. J. Exp. Med. 172:1091-1100.

160. Bretscher, P., and M. Cohn. 1970. A theory of self-nonself discrimination. Science 169:1042-1049.

161. Schwartz, R. H. 1990. A cell culture model for T lymphocyte clonal anergy. Science 248:1349-1356.

162. Davis, L.S., M.C. Wacholtz, and P.E. Lipsky. 1988. The induction of T cell unresponsiviemess by rapidly modulating CD3. J. Immunol. 142:1084-1094.

163. Jensen, P.E., C.W. Pierce, and J.A. Kapp. 1984. Regulatory mechanisms in immune responses to heterologous insulins. II. Suppressor T cell activation associated with nonresponsiveness in H-2^b mice. J. Exp. Med. 160:1012-1026.

164. Nagarkatti, P.S., M. Nagarkatti, L.W. Mann, L.A. Jones, and A.M. Kaplan. 1988. Characterization of and endogenous Lyt 2^+ T-suppressor-cell population reguating autoreactive T cells *in vitro* and *in vivo*. Cell. Immunol. 112:64-77.

165. Atassi, M.Z., K.-H. Ruan, K. Jinnai, M. Oshima, and T. Ashizawa. 1992. Epitope-specific suppression of antibody-response in experimental autoimmune myasthemia gravis by a monomethoxypolyethylene glycol conjugate of a myasthenogenic synthetic peptide. Proc. Natl. Acad. Sci. USA 89:5825-5856.

166. Finberg, R., S.J. Burakoff, B. Benacerraf, and M.I. Greene. 1979. The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. II. Evidence for antigen-specific suppressor T cells. J. Immunol. 123:1210-1214.

167. Adorini, L., A. Miller, and E.E. Sercarz. 1979. The fine specificity of regulatory T cells. I. Hen egg-white lysozyme-induced suppressor T cells in a genetically nonresponder mouse strain do not recognize a closely related immunogenic lysozyme. J. Immunol. 122:871-876.

168. Roser, D.J. 1989. Cellular mechanisms in neonatal and adult tolerance. Immunol. Rev. 107:179-202.

169. Dorf, M.E., and B. Benacerraf. 1984. Suppressor cells and immunoregulation. Ann. Rev. Immunol. 2:127-158.

170. Bloom, B.R., P. Salgame, and B. Diamond. 1992. Revisiting and revising suppressor T cells. Immunol. Today 13:131-142.

171. Binz, H., and H. Wigzell. 1978. Induction of specific immune unresponsiveness with purified mixed leukocyte culture-activated T lymphoblasts as autoimmunogen. III. Proof for the existence of autoanti-idiotypic killer T cells and transfer of suppression to normal syngeneic recipients by T or B lymphocytes. J. Exp. Med. 147:63-76.

172. Weinberger, J.Z., R.N. Germain, S.-T. Ju, M.I. Greene, B. Benacerraf, and M.E. Dorf. 1979. Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. J. Exp. Med. 150:761-776.

173. Dorsch, S., and B. Roser. 1982. Suppressor cells in transplantation tolerance. II. Identification and probable mode of action of chimeric suppressor T cells. Transplantation 33:524-529.

174. Lohse, A.W., E. Mor, T. Reshef, K.-H.M. zum Buschenfelde, and I.R. Cohen. 1990. Inhibition of the mixed lymphocyte reaction by T cell vaccination. Eur. J. Immunol. 20:2521-2524.

175. Dalme, N.K., N, Mohagheghpour, and E.G. Engleman. 1984. Soluble antigen primed inducer T cells activate antigen specific suppressor T cells in the absence of antigen pulsed accessory cells. Phenotypic definition of suppressor inducer and suppressor effector cells. J. Immunol. 132:644-700.

176. Heuer, J., and E. Kolsch. 1986. Selective elimination through a cytolytic mechanism of bovine serum albumin-specific T helper lymphocytes by T suppressor cells with the same antigen specificity. Eur. J. Immunol. 16:400-404.

177. Sun, D., Y. Qin, J. Chluba, J.T. Epplen, and H. Wekerle. 1988. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interaction. Nature 332:843-845.

178. Lider, O., T. Reshef, E. Beraud, A. Ben-Nun, and I.R. Cohen. 1988. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science 239:181-183.

179. Howell, M.D., S.T. Winters, T. Olee, H.C. Powell, D.J. Carlo, and S.W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. Science 246:668-670.

180. Offner, H., G.A. Hashim, and A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. Science 251:430-432.

181. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541-544.

182. Batchelor, J.R., G. Lombardi, and R.I. Lechler. 1989. Speculations on the specificity of suppression. Immunol. Today 10:37-40.

183. Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman, and S. Abrignani. 1988. T cells can present antigens such as HIV gp120 targeted to their own surface molecules. Nature 334:530-532.

184. Lakey, E.K., E. Margoliash, G. Flouret, and S.K. Pierce. 1986. Peptides related to the antigenic determinant block T cell recognition of the native protein as processed by antigen-presenting cells. Eur. J. Immunol. 16:721-727.

185. Babbitt, B.P., G. Matsueda, E. Haber, E.R. Unanue, and P.M. Allen. 1986. Antigenic competion at the level of peptide-Ia binding. Proc. Natl. Acad. Sci. USA 83:4509-4513.

186. Adorini, L., S. Muller, F. Cardinaux, P.V. Lehmann, F. Falcioni, and Z.A. Nagy. 1988. In vivo competition between self peptides and foreign antigens in T-cell activation. Nature 334:623-625.

187. Gautam, A.M., and P. Glynn. 1990. Competition between foreign and self proteins in antigen presentation: ovalbumin can inhibit activation of myelin basic protein-specific T cells. J. Immunol. 144:117-1180.

188. Benichou, G., P.A. Takizawa, P.T. Ho, C.C. Killion, C.A. Olson, M. McMillan, and E.E. Sercarz. 1990. Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides. J. Exp. Med. 172:1341-1346.

189. Agrawal, B., M. Manickasundari, E. Fraga, and B. Singh. 1991. T cells that recognize peptide sequences of self MHC class II molecules exist in syngeneic mice. J. Immunol. 147:383-393.

190. Lorenz, R.G., and P.M. Allen. 1988. Direct evidence for functional self-protein/Ia-molecule complexes in vivo. Proc. Natl. Acad. Sci. USA 85:5220-5223.

191. Bikoff, E.K., H. Yu, and L.A. Eckhardt. 1988. T cell recognition of endogenous IgG_{2a} expressed in B lymphoma cells. Eur. J. Immunol. 18:341-348.

192. Weiss, S., and B. Bogen. 1989. B-lymphoma cells process and present their endogenous immunoglobulin to major histocompatibility complex-restricted T cells. Proc. Natl. Acad. Sci. USA 86:282-286.

193. Wekerle, H., M. Schwab, C. Linington, and R. Meyermann. 1986. Antigen presentation in the peripheral nervous system: Schwann cells present endogenous myelin autoantigens to lymphocytes. Eur. J. Immunol. 16:1551-1557.

194. Schild, H., O. Rotzschke, H. Kalbacher, and H.-G. Rammensee. 1990. Limit of T cell tolerance to self proteins by peptide presentation. Science 247:1587-1589.

195. MacNeil, D., E. Fraga, and B. Singh. Characterization of murine T cell response to peptides of self T cell receptor β chains. (submitted)

196. Gammon, G., and E. Sercarz. 1989. How some T cells escape tolerance induction. Nature 342:183-185.

197. Benichou, G., and E. Sercarz. 1990. The presentation of self-peptides: tolerance and competition. Intern. Rev. Immunol. 6:75-88.

198. Sinha, A.A., M.T. Lopez, and H.O. McDevitt. 1990. Autoimmune diseases: the failure of self tolerance. Science 248:1380-1387.

199. Singh, V.K., H.K. Karla, K. Yamaki, T. Abe, L.A. Donoso, and T. Shinohara. 1990. Molecular mimicry between a uveitopathogenic site of S-antigen and viral peptides: induction of experimental autoimmune uveitis in Lewis rats. J. Immunol. 144:1283-1287.

200. Castano, L., and G.S. Eisenbarth. 1990. Type-1 diabetes: a chronic autoimmune disease of human, mouse, and rat. Annu. Rev. Immunol. 8:647-679.

201. Elliott, R.B., and J.M. Martin. 1984. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? Diabetologia 26:297-299.

202. Gerling, I., C. Nejman, and N.K. Chatterjee. 1988. Effect of Coxsackievirus B4 infection in mice on expression of 64,000-M_r autoantigen and glucose sensitivity of islets before development of hyperglycemia. Diabetes 37:1419-1421.

203. Wicker, L.S., B.J. Miller, L.Z. Coker, S.E. McNally, S. Scott, Y. Mullen, and M.C. Appel. 1987. Genetic control of diabetes and insulitis in the nonobese diabetic (NOD) mouse. J. Exp. Med. 165:1639-1654.

204. Prochazka, M., E.H. Leiter, D.V. Serreze, and D.L. Coleman. 1987. Three recessive loci required for insulin-dependent diabetes in nonobese diabetic mice. Science 237:286-289.

205. Hattori, M., J.B. Buse, R.A. Jackson, L. Glimcher, M.E. Dorf, M. Minami, S. Makino, K. Moriwake, H. Kuzuya, H. Imura, W.M. Strauss, J.G. Seidman, and G.S. Eisenbarth. 1986. The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. Science 231:733-735.

206. Acha-Orbea, H., and H.O. McDevitt. 1987. The first external domain of the nonobese diabetic mouse class II I-A β chain is unique. Proc. Natl. Acad. Sci. USA 84:2435-2439.

207. Todd, J.A., J.I. Bell, and H.O. McDevitt. 1987. HLA-DQβ gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329:599-603.

208. Serreze, D.V., E.H. Leiter, S.M. Worthen, and L.D. Shultz. 1988. NOD marrow stem cells adoptively transfer diabetes to resistant (NOD x NON)F1 mice. Diabetes 37:252-255.

209. Wicker, L.S., B.J. Miller, A. Chai, M. Terada, and Y. Mullen. 1988. Expression of genetically determined diabetes and insulitis in the nonobese diabetic (NOD) mouse at the level of bone marrow-derived cells. Transfer of diabetes and insulitis to nondiabetic (NOD x B10)F1 mice with bone marrow cells from NOD mice. J. Exp. Med. 167:1801-1810.

210. Schwizer, R.W., E.H. Leiter, and R. Evans. 1984. Macrophage-mediated cytotoxicity against cultured pancreatic islet cells. Transplant. 37:539-543.

211. Ogawa, M., T. Maruyama, T. Hasegawa, T. Kanaya, F. Kobayashi, Y. Tochino, and H. Uda. 1985. The inhibitory effect of neonatal thymectomy on the incidence of insulitis in non-obese diabetes (NOD) mice. Biomed. Res. 6:103-105.

212. Kioke, T., Y. Itoh, T. Ishii, I. Ito, K. Takabayashi, N. Maruyama, H. Tomioka, and S. Yoshida. 1987. Preventive effect of monoclonal anti-L3T4 antibody on development of diabetes in NOD mice. Diabetes 36:539-541.

213. Wang, Y., L. Hao, R.G. Gill, and K.J. Lafferty. 1987. Autoimmune diabetes in NOD mouse is L3T4 T-lymphocyte dependent. Diabetes 36:535-538.

214. Wicker, L.S., B.J. Miller, and Y. Mullen. 1986. Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. Diabetes 35:855-860.

215. Reich, E.-P., R.S. Sherwin, O. Kanagawa, and C.A. Janeway, Jr. 1989. An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes. Nature 341:326-328.

216. Zamvil, S.S., and L. Steinman. 1990. The T lymphocyte in experimental allergic encephalomyelitis. Ann. Rev. Immunol. 8:579-621.

217. Paliard, X., S.G. West, J.A. Lafferty, J.R. Clements, J.W. Kappler, P. Marrack, and B.L. Kotzin. 1991. Evidence for the effects of a superantigen in rhematoid arthritis. Science 253:325-329.

218. Wucherpfennig, K.W., J. Newcombe, H. Li, C. Keddy, M.L. Cuzner, and D.A. Hafler. 1992. T cell receptor Vα-Vβ repertoire and cytokine gene expression in active multiple sclerosis lesions. J. Exp. Med. 175:993-1002.

219. Steinman, L., J.R. Oksenberg, and C.C.A. Bernard. 1992. Association of susceptibility to multiple sclerosis with TCR genes. Immunol. Today 13:49-55.

220. Nakano, N., H. Kikutani, H. Nishimoto, and T. Kishimoto. 1991. T cells receptor V gene usage of islet β cells-reactive T cells is not restricted in non-obese diabetic mice. J. Exp. Med. 173:1091-1097.

221. Candeias, S., J. Katz, C. Benoist, D. Mathis, and K. Haskins. 1991. Islet-specific T-cell clones from nonobese diabetic mice express heterogeneous T-cell receptors. Proc. Natl. Acad. Sci. USA 88:6167-6170.

222. Walters, S.H., J.J. O'neil, D.T. Melican, and M.C. Appel. 1992. Multiple TCR Vβ usage by infiltrates of young NOD mouse islets of Langerhans. Diabetes 41:308-312.

223. Shizuru, J.A., C. Taylor-Edwards, A. Livingstone, and C.G. Fathman. 1991. Genetic dissection of T cell receptor Vβ gene requirements for spontaneous murine diabetes. J. Exp. Med. 174:633-638.

224. Lipes, M.A., R.G. Fenton, L. Zhou, J.F. Seidman, and G.S. Eisenbarth. 1989. Autoimmunity occurs in transgenic T-cell

receptor (TCR) beta gene non-obese diabetic (NOD) mice. Clin. Res. 37:572A.

225. Danska, J. 1992. Molecular analysis of T cell receptors resident in the early pancratic infiltates of NOD mice. Annual Meeting of the Canadian Society of Immunologists. Abstract 1.4

226. Boitard, C., A. Bendelac, M.F. Richard, C. Carnaud, and J.F. Bach. 1988. Prevention of diabetes in nonobese diabetic mice by anti-I-A monoclonal antibodies: transfer of protection by splenic T cells. Proc. Natl. Acad. Sci. USA 85:9719-9723.

227. Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. Proc. Natl. Acad. Sci. USA 88:3088-3091.

228. Sadelain, M.W.J., H.-Y. Qin, J. Lauzon, and B. Singh. 1990. Prevention of type-1 diabetes in NOD mice by adjuvant immunotherapy. Diabetes 39:583-589.

229. Qin, H.-Y., M.W.J. Sadelain, C. Hitchon, J. Lauzon, and B. Singh. Complete Freund's adjuvant induced T cells prevent the development and adoptive transfer of diabetes in nonobese diabetic (NOD) mice. (submitted).

230. Yagi, H. M. Matsumoto, Y. Kishimoto, S. Makino, and M. Harada. 1991. Possible mechanism of the preventive effect of BCG against diabetes mellitus in NOD mouse. II. Suppression of pathogenesis by macrophage transfer from BCG- vaccinated mice. Cell. Immunol. 138:142-149.

231. Wucherpfennig, K.W., H.L. Weiner, and D.A. Hafler. 1991. T-cell recognition of myelin basic protein. Immunol. Today 12:277-282.

232. Fritz, R.B., M.J. Skeen, C.-H.J. Chou, M. Garcia, and I.K. Egorov. 1985. Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. J. Immunol. 134:2328-2332.

233. Traugott, U., D.E. McFarlin, and C.S. Raine. 1986. Immunopathology of the lesion in chronic relapsing experimental autoimmune encephalomyelitis in the mouse. Cell. Immunol. 99:395-410.

234. Gonatas, N.K., and J.C. Howard. 1974. Inhibition of experimental allergic encephalomyelitis in rats severely depleted of T cells. Science 186:839-841.

235. Waldor, M.K., S. Sriram, R. Hardy, L.A. Herzenberg, L.A. Herzenberg, L. Lanier, M. Lim, and L. Steinman. 1985. Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. Sceince 227:415-417.

236. Pettinelli, C.B., and D.E. McFarlin. 1981. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after *in vitro* activation of lymph node cells by myelin basic protein: requirement for Lyt $1^{+}2^{-}$ T lymphocytes. J. Immunol. 127:1420-1423.

237. Ben-Nun, A., H. Wekerle, and I.R. Cohen. 1981. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur. J. Immunol. 11:195-199.

238. Zamvil, S.S., D.J. Mitchell, N.E. Lee, A.C. Moore, M.K. Waldor, K. Sakai, J.B. Rothbard, H.O. McDevitt, L. Steinman, and H. Acha-Orbea. 1988. Predominant expression of a T cell receptor Vβ gene subfamily in autoimmune encephalomyelitis. J. Exp. Med. 167:1586-1596.

239. Wraith, D.C., H.O. McDevitt, L. Steinman, and H. Acha-Orbea. 1989. T cell recognition as the target for immune intervention in autoimmune disease. Cell 57:709-715.

240. Burns, F.R., X. Li, N. Shen, H. Offner, Y.K. Chou, A.A. Vandenbark, and E. Herber-Katz. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility

complex and encephalitogenic determinants being recognized are different. J. Exp. Med. 169:27-30.

241. Acha-Orbea, H., D.J. Mitchell, L. Timmermann, D.C. Wraith, G.S. Tausch, M.K. Waldor, S.S. Zamvil, H.O. McDevitt, and L. Steinmann. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell 54:263-273.

242. Urban, J.L., V. Kumar, D.H. Kono, C. Gomez, S.J. Horvath, J. Clayton, D.G. Ando, E.E. Sercarz, and L. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell 54:577-592.

243. Desquenne-Clark, L., T.R. Esch, L. Otvos, Jr., and E. Heber-Katz. 1991. T-cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis. Proc. Natl. Acad. Sci. USA 88:7219

244. Kawano, Y.-I., Y. Sasamoto, S. Kotake, S.R. Thurau, B. Wiggert, and I. Gery. 1991. Trials of vaccination against experimental autoimmune uveoretinitis with a T-cell receptor peptide. Curr. Eye Res. 10:789-795.

245. Mosmann, T.R., and R.L. Coffman. 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann. Rev. Immunol. 7:145-173.

246. Bendelac, A., and R.H. Schwartz. 1991. Th0 cells in the thymus: the question of T-helper lineages. Immunol. Rev. 123:168-187.

247. Salgame, P., J.S. Abrams, C. Clayberger, H. Goldstein, J. Convit, R.L. Modlin, and B.R. Bloom. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science 254:279-282.

248. DelPrete, G.F., M. DeCarli, M. Ricci, and S. Romagnani. 1991. Helper activity for immunoglobulin synthesis of T helper Type 1 (Th1) and Th2 human T cell clones: the help of Th1

clones is limited by their cytolytic capacity. J. Exp. Med. 174:809-813.

249. Mosmann, T.R., J.H. Schumacher, N.F. Street, R. Budd, A. O'Garra, T.A.T. Fong, M.W. Bond, K.W.M. Moore, A. Sher, and D.F. Fiorentino. 1991. Diversity of cytokine synthesis and function of mouse $CD4^+$ T cells. Immunol. Rev. 123:209-229.

250. Gajewski, T.F., J. Joyce, and F.W. Fitch. 1989. Antiproliferative effect of IFN-γ in immune regulation. III. Differential selection of Th1 and Th2 murine helper T lymphocyte clones using rcombinant IL-2 and recombinant IFN-γ. J. Immunol. 143:15-22.

251. Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348-2357.

252. Scott, P., P. Natovitz, R.L. Coffman, E. Pearce, and A. Sher. 1988. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protictive immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. J. Exp. Med. 168:1675-1684.

253. Muller, I., and J.A. Louis. 1989. Immunity to experimental infection with *Leishmania major*: generation of protective $L3T4^+$ T cell clones recognizing antigen(s) associated with live parasites. Eur. J. Immunol. 19:863-871.

254. Ashbridge, K.R., B.T. Backstrom, H.-X. Li, T. Vikerfors, D.R. Englebretsen, D.R.K. Harding, and J.D. Watson. 1992. Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of *Mycobacterium tuberculosis*: evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity responses. J. Immunol. 148:2248-2255.

255. Murray, J.S., J. Madri, J. Tite, S.R. Carding, and K. Bottomly. 1989. MHC control of $CD4^+$ T cell subset activation. J. Exp. Med. 170:2135-2140.

256. Jerne, N.K. 1974. Towards a network theory of the immune system. Ann. Immunol. (Inst. Pasteur) 125C:373-389.

257. Holmberg, D., S. Forsgren, F. Ivars, and A. Coutinho. 1984. Reactions among IgM antibodies derived from normal, neonatal mice. Eur. J. Immunol. 14:435-441.

258. Lundkvist, I., A. Coutinho, F. Varela, and D. Holmberg. 1989. Evidence for a functional idiotypic network among natural antibodies in normal mice. Proc. Natl. Acad. Sci. USA 86:5074-5078.

259. Kourilsky, P., G. Chaouat, C. Rabourdin-Combe, and J.-M. Claverie. 1987. Working principles in the immune system implied by the "peptidic self" model. Proc. Natl. Acad. Sci. USA 84:3400-3404.

260. Pereira, P., E.-L. Larsson, L. Forni, A. Bandeira, and A. Coutinho. 1985. Natural effector T lymphocytes in normal mice. Proc. Natl. Acad. Sci. USA 82:7691-7695.

261. Kilgannon, P. 1991. T cell receptor repertoire to poly-18 and Mls-1a: parallels between a peptide antigen and superantigen recognition. PhD Thesis. Univ. of Alberta, Edmonton, Alberta.

CHAPTER II

Inhibition of superantigen recognition by peptides of the variable region of the T cell receptor β chain[1]

Introduction

The antigen receptor of T lymphocytes (TCR) recognizes antigen in a denatured or processed form (1) in the context of MHC (2). The TCR is a glycoprotein heterodimer composed of α and β chains or γ and δ chains, which are associated with the CD3 complex. Each chain has a variable N-terminal domain encoded by V, J or V, D, J gene regions (3,4). The V and J regions of both the α and β chains probably contribute to MHC restriction and antigen specificity (5). However, in some cases one chain appears to contribute more than the other in antigen specificity, eg. $V_{\alpha 11}$ with pigeon cytochrome c (6,7) and $V_{\beta 8}$ with myelin basic protein (8-10). This is especially true for the endogenous superantigen, minor lymphocyte stimulating locus (Mls), which stimulates nearly all T cells bearing a particular V_{β} region in mice. Mls has been identified as an endogenous mouse mammary tumor virus (11). T cells bearing $V_{\beta 3}$, $V_{\beta 6}$, $V_{\beta 8.1}$, and $V_{\beta 9}$ are stimulated by one or more forms of Mls (12-

---

[1] A version of this chapter has been published. MacNeil, Fraga, and Singh 1992. European Journal of Immunology 22:937-941.

15) in the context of a non-polymorphic region of the class II MHC molecules (16).

In order to identify putative sites of interaction of TCR-V_β region and Mls, we have taken advantage of a panel of five Mls-1^a reactive T cell hybridomas, four of which are $V_{\beta 6}$ positive and one of which is $V_{\beta 8}$ positive. These cells also respond to a nominal antigen Poly-18, Poly $EYK(EYA)_5$, in the context of I-A^d (17), and its synthetic peptide fragments without processing (17, 18). We tested the response of these T cell hybridomas in the presence of peptides of the TCR β chain which are derived from the variable region of the $V_{\beta 6}$ gene segment. Based on homology to immunoglobulins, the peptides correspond to regions which comprise the solvent-exposed β-sheet on the TCR, outside the regions which interact with antigen or the α chain (19). We show that three peptides corresponding to amino acid positions 1 to 20, 48 to 75 and 58 to 75 of the $V_{\beta 6}$ peptide sequence can interfere with activation of these $V_{\beta 6}^+$ hybridomas by Mls-1^a bearing spleen cells. At the same time these peptides do not interfere with antigen recognition by $V_{\beta 6}^+$ hybridomas or with Mls-1^a recognition by the $V_{\beta 8}^+$ hybridoma. The sequences 1 to 20 and 58 to 75 are proposed to lie outside the putative binding domain of processed antigen (19), indicating that recognition by TCR of Mls is different from the classical MHC restricted recognition of processed antigen. These results suggest that the recognition of superantigen/class II MHC by T cells can be inhibited by peptides related to the binding region of the TCR.

Materials and Methods

Mice

BALB/cCr mice (H-2^d, Mls-1^b, 2^a) were bred at the Ellerslie Animal Farm of the University of Alberta. DBA/2J (H-2^d, Mls-1^a, 2^a), CBA/CaJ (H-2^k, Mls-1^b, 2^b), CBA/J (H-2^k, Mls-1^a, 2^a), C3H/HeJ (H-2^k, Mls-1^b, 2^a), and AKR/J (H-2^k, Mls-1^a, 2^b) mice were purchased from the Jackson Laboratory, Bar Harbor, ME.

T cell hybridomas

Poly-18, Poly EYK(EYA)$_5$-specific, I-A^d restricted T cell hybridomas were generated in this laboratory as described by fusion of BALB/C derived Poly-18-specific T cell lines to either BW5147 thymoma or the BW5147$\alpha^-\beta^-$ thymoma using a standard PEG fusion protocol (17, 20). T cell line(s) were derived from BALB/c mice immunized with Poly-18 antigen emulsified in CFA (17). T cell hybridomas were screened for IL-2 production as described below.

Peptides

Peptides were prepared by the Merrifield solid-phase technique on a Beckman 990C Peptide Synthesizer (Palo Alto, CA), as previously described (17, 18). All amino acids were protected at the a amino position with the Boc group and the following side chain blocking groups were used: Glu(O-benzyl, Asp(O-benzyl), Arg(tosyl), His(tosyl), Tyr(2-bromobenzoxycarbonyl), Lys(2-chlorobenzoxycarbonyl),Gln (4,4'-dimethoxybenzhydryl), Thr(benzyl), and Ser(benzyl). Dicyclohexyl-carbodiimide was used

as the activating agent and double couplings of 2 h each were performed at each step with 2.5 equivalents of Boc amino acids. The Boc groups were removed at each cycle of the synthesis by treatment with 25% trifluoracetic acid (TFA) in methylene chloride (v/v) for 20 min and neutralization was carried out by treatment with 5% diisoprpoyl ethylamine in methylene chloride (v/v). The cleavage of the peptide from the resin support and removal of the blocking groups was carried out in hydrofluoric acid (HF) at 0°C for 2 h with 10% anisol and 0.1% ethane dithiol as scavengers. After removal of the HF under vacuum, the resin was washed with ether and the peptide extracted with TFA. The TFA was evaporated to a small volume and the peptide was precipitated with ether. After precipitation the peptides were dissolved in water and lyophilized. The peptides were purified by HPLC on a C-18 reverse phase semipreparative SynChropak RP-P column (Synchrom, Inc., Linden, IN). Peptides were eluted with a linear gradient from water to acetonitrile (1.37% acetonitrile/min). Fractions were pooled and lyophilized. Amino acid analyses were performed on the purified peptides by using a Beckman System 6300 amino acid analyzer. For this purpose samples were hydrolysed in 6 N HCl containing 0.1% phenol in sealed evacuated tubes for 20 h at 110°C. All peptides gave acceptable ratios of the various amino acids. For functional assays, peptides were dissolved in saline by adjusting pH to 7.2 with 0.1 N NaOH and were sterilized by filtration through a 0.22 μm filter.

IL-2 assay

For stimulation with Mls, T hybridoma cells ($1x10^5$) were cultured with $1x10^6$ or $5x10^5$ irradiated (3000 rad) spleen cells of DBA/2 mice (H-2^d, Mls-1^a, Mls-2^a) in the presence or absence of competing peptide in 250 µl of culture medium (RPMI 1640 (Gibco Labs., Grand Island, NY) supplemented with 10% fetal bovine serum (Bocknek Labs., Canada), 10 mM Hepes, 2 mM glutamine, $5x10^{-5}$ M 2-ME, and 1 U/ml penicillin-streptomycin), in each well of a 96-well microtitre plate (Flow Labs. Inc., McLean, VA). For stimulation with antigen, T hybridoma cells ($1x10^5$) were cultured with $1x10^6$ irradiated (3000 rad) spleen cells of BALB/C mice (H-2^d, Mls-1^b, Mls-2^a), and 20 µM $(EYA)_5$ in the presence or absence of competing peptide in 250 µl of culture medium. Competing peptides were used at 80 µg/mL, approximately 75 µM. After 21-24 h, supernatants were collected and assayed for IL-2 content in secondary cultures of the IL-2 dependent CTL-L cell line. Cells were cultured in 50% or 25% of test supernatant for 24 h, then pulsed with 37 kBq of [^{3}H]dThd (NEN Dupont, Boston, MA) for 12-18 h. Incorporation of [^{3}H]dThd was measured using a liquid scintillation counter (LKB Instruments, Gaithersburg, MD).

Results and Discussion

T cell hybridomas B2, B4, B5, and B11 have been shown to be $V_{\beta 6}^+$, and B9 has been shown to be $V_{\beta 8}^+$ by Northern analysis and fluorescence activated cell scanning with monoclonal antibodies 44.22.1 and KJ16 (17, Kilgannon, et. al., submitted). The above T hybridomas are specific for Poly-18, and restricted to I-A^d (17). B5, B9, and B17 also respond to two peptides derived from Poly-18, EYK$(EYA)_3$EYK, and $(EYA)_5$; B4 and B11 also respond to $(EYA)_5$ in the context of I-A^d. Crossreactivity of these hybridomas for Mls-1^a is demonstrated in Table 2.1. As with most Mls reactive T cell clones and hybridomas (21), antibodies to I-A and to I-E can partially inhibit response to Mls and anti-I-A mAb with anti-I-E mAb block response totally.

In order to identify which regions of the β chain of the TCR are involved in recognition of Mls, we tested the ability of peptides derived from the sequence of $V_{\beta 6}$ to block recognition of Mls-1^a. The peptides chosen in this study are $V_{\beta 6}$(1-20), $V_{\beta 6}$(32-48), $V_{\beta 6}$(39-60), $V_{\beta 6}$(48-75), and $V_{\beta 6}$(58-75) (see Table 2.2). The corresponding sequences are proposed to lie outside the putative antigen binding domain, except for a considerable portion of $V_{\beta 6}$(48-75) and of $V_{\beta 6}$(39-60) which contain Complementarity Determining Region 2 (CDR2), an area proposed to contact MHC molecules in antigen presentation (19). In Fig. 2.1. are shown the effects of the peptides on the recognition of Mls-1^a by $V_{\beta 6}^+$ T cell hybridomas. Peptide fragments $V_{\beta 6}$(1-20), $V_{\beta 6}$(48-75), and $V_{\beta 6}$(58-

75) can significantly inhibit the response by the $V_{\beta 6}^+$ hybridomas to varying degrees. The peptide corresponding to residues 39 to 60 inhibits the response of one of the hybridomas, B11, but does not inhibit the other $V_{\beta 6}^+$ hybridomas. This might reflect a difference in affinity of the TCR of B11 for Mls-1^a from that of the other hybridomas, since B11 differs from the other hybridomas in J_β and V_α usage. The same peptides do not inhibit the response to Mls-1^a by the $V_{\beta 8}^+$ hybridoma, B9 (Fig. 2). The peptide corresponding to residues 32 to 48 enhances the response to Mls-1^a. The increased response in the presence of $V_{\beta 6}$(32-48) is difficult to explain. This peptide is not acting as a mitogen, since $V_{\beta 6}$(32-48) does not stimulate B5 in the presence of irradiated spleen cells from BALB/c (Mls-1^b) (data not shown). It is possible that this peptide may stabilize MHC molecules and thus increase the density of Mls/MHC complexes on the cell surface of the APC, leading to a stronger stimulation of the T cell hybridomas. At the concentrations tested, 75 μM, the peptides are not toxic to the responding hybridomas or indicator cell line, CTL-L (data not shown). Thus peptides corresponding to certain regions of the TCR-$V_\beta 6$ protein sequence are able to inhibit the recognition of Mls-1^a by T cell hybridomas bearing the $V_{\beta 6}$ segment of the TCR, but not the $V_{\beta 8}$ segment.

Although we are comparing peptides of different lengths, the length of a peptide should not have a great effect on its inhibitory activity. For example, the largest peptide, $V_{\beta 6}$(48-75), does not in all cases inhibit to a greater degree than the shorter peptide $V_{\beta 6}$(58-75),

(see Fig. 2.1d versus Fig. 2.1a, c). Secondly, two peptides of similar length, $V_{\beta 6}$(32-48) and $V_{\beta 6}$(58-75), 16 and 17 amino acids respectively, have opposite effects on the recognition of Mls-1^a by the $V_{\beta 6}^+$ T cell hybridomas. Thus, the sequence of amino acids is more important than the number of amino acids of the peptide in determining the peptides' inhibitory activity.

The peptides were tested for their effect on antigen recognition by one of the $V_{\beta 6}^+$ hybridomas to determine the specificity of this inhibition and to determine if the peptides are able to associate with class II molecules at the antigen binding groove. Peptide fragments of Poly-18 do not require processing to be presented by I-A^d to these T cell hybridomas. To avoid complications due to processing of antigen, $(EYA)_5$ was used to stimulate the T cell hybridoma, B11, in the presence of $V_{\beta 6}$ peptides. As shown in Fig. 2.3, the three inhibitory peptides had no effect on the response to 20 μM of $(EYA)_5$ by B11. This might be because the peptides lack the tertiary configuration of the antigen/ MHC binding site of the TCR. The fact that the peptides which partially inhibited the recognition of Mls-1^a were not able to inhibit the recognition of $(EYA)_5$ by the same T cell hybridoma is consistent with the hypothesis that interaction between TCR, class II, and Mls is different from that of TCR, class II, and antigen. It also implies that the site of interaction of the competing peptides is not between the TCR and MHC molecules, rather between the TCR and Mls. Because B11 can respond to antigen in the presence of $V_{\beta 6}$ peptides, the

effect of the peptides on the response to Mls-1^a cannot be due to toxicity or a non-specific blocking of stimulation. Thus the effect of the $V_{\beta 6}$ peptides is specific to the recognition of Mls-1^a by $V_{\beta 6}^+$ T cell hybridomas.

These results suggest two important findings. First, since the $V_{\beta 6}$ peptides do not inhibit the response to $(EYA)_5$, the recognition of Mls and that of nominal antigen may differ in sites of interaction and affinity with the TCR. Based on homology of the TCR to immunoglobulins, (19), the two sequences 1 - 20 and 58 - 75 are proposed to lie outside the putative binding domain for processed antigen. These results are in agreement with recent findings. Choi *et al.* (22) have shown by transfection of human TCR α and β genes into murine T cell hybridoma, that residues 67 to 77 of $V_{\beta 13.2}$ are important in stimulation by the superantigen, staphylococcal enterotoxin C. Pullen *et al.* (23) have shown by site directed mutagenesis of the $V_{\beta 8.2}$ gene, that residues 22, 70, and 71 of $V_{\beta 8.1}$ are involved in recognition of Mls-1^a. These residues are also in or near the two sequences 1 to 20 and 58 to 75. Pullen *et al.* (24) have also shown that mutations of $V_{\beta 8.1}$ which affect the response to T cell hybridomas to Mls-1^a do not affect the response to nominal antigen, cOVA in the context of I-A^d. Our findings also suggest that the TCR has two distinct binding sites, one for nominal antigen, and another for superantigens

Second, peptide fragments of the variable region of the TCR are able to inhibit the response of T cell hybridomas to the superantigen, Mls. Ours is the first demonstration that peptides of the TCR can influence the functional response of T cells to superantigens. These data have implications in the potential regulatory role of peptides of the TCR in immune responses. Deletion in the thymus is the major mechanism of tolerance induction to superantigens, Mls (25) as well as SEB (26), presumably by the presentation of the superantigen to reactive T cells by dendritic cells in the thymus (27). If the majority of T cells expressing a particular V_β are deleted, there is the possibility that fragments of the TCR variable region are presented by class II and class I bearing cells following the degradation of the TCR expressed by the deleted T cells. Therefore, the potential exists that T cells may interact with peptides of the TCR *in vivo*, resulting in the selection of a T cell repertoire reactive with various V_β peptides. The regulation of immune responses by immunization with peptides of the TCR variable region has been demonstrated in the EAE system (28 - 30) and the potential of this approach in the regulation of the immune response is investigated in Chapter IV.

Table 2.1 Mls-1[a] specificity of T cell hybridomas

Stimulator cells	Monoclonal Antibody[a)]	Responding T cell hybridoma B2	B4	B5	B11	B9
none	none	0.5 ± 0.2	3.1 ± 1.2	0.4 ± 0.1	2.4 ± 0.6	2.9 ± 1.0
BALB/C	none	0.3 ± 0.1	2.7 ± 0.8	0.3 ± 0.1	5.6 ± 1.1	5.4 ± 2.4
DBA/2J	none	1.7 ± 0.2	117.1 ± 48.4	7.2 ± 1.3	28.1 ± 2.1	65.5 ± 5.2
	17.3.3S	0.5 ± 0.1	N.D.	1.2 ± 0.1	16.5 ± 1.6	N.D.
	10.3.6.2	0.9 ± 0.4	N.D.	3.9 ± 0.0	28.9 ± 7.3	N.D.
	MKD6	0.3 ± 0.1	N.D.	0.6 ± 0.3	2.8 ± 0.3	N.D.
	GK1.5	0.3 ± 0.0	N.D.	0.3 ± 0.0	0.3 ± 0.0	N.D.
CBA/CaJ	none	0.3 ± 0.1	2.6 ± 0.2	0.2 ± 0.1	6.9 ± 1.5	2.6 ± 1.3
C3H/HeJ	none	0.3 ± 0.1	2.8 ± 0.2	0.8 ± 0.5	8.0 ± 2.0	N.D.
CBA/J	none	2.7 ± 0.4	221.3 ± 12.6	20.0 ± 6.0	90.5 ± 16.0	52.1 ± 6.8
	17.3.3S	0.3 ± 0.1	N.D.	0.3 ± 0.0	17.3 ± 4.1	N.D.
	10.3.6.2	0.4 ± 0.2	N.D.	0.4 ± 0.1	21.6 ± 2.2	N.D.
	MKD6	3.3 ± 0.1	N.D.	20.8 ± 1.1	51.3 ± 0.7	N.D.
	GK1.5	0.3 ± 0.0	N.D.	0.3 ± 0.1	0.3 ± 0.1	N.D.
	17.3.3S & 10.3.62	0.3 ± 0.0	N.D.	0.3 ± 0.1	0.4 ± 0.8	N.D.
AKR/J	none	8.9 ± 1.4	107.6 ± 8.6	35.1 ± 6.4	82.7 ± 4.6	N.D.
	17.3.3S	0.3 ± 0.1	N.D.	0.5 ± 0.1	17.9 ± 5.0	N.D.
	10.3.6.2	0.4 ± 0.0	N.D.	0.6 ± 0.1	10.8 ± 3.2	N.D.
	MKD6	10.6 ± 1.0	N.D.	45.8 ± 6.9	59.0 ± 0.4	N.D.
	GK1.5	0.3 ± 0.1	N.D.	1.0 ± 0.4	0.8 ± 0.0	N.D.
	17.3.3S & 10.3.62	0.3 ± 0.1	N.D.	0.3 ± 0.0	1.4 ± 0.6	N.D.

a) Specificites are as follows: 10.3.6.2 (anti-I-A^k), 17.3.3S (anti-I-E^k), MKD6 (anti-I-A^d), and GK1.5 (anti-CD4).

Table 2.2 Sequences of $V_\beta 6$ peptides

Peptide[a)]	Sequence[b)]
1 - 20	GGIITQTPKFLIGQEGQKLT
32 - 48	MYWYRQDSGKGLRLIYY
39 - 60	DSGKGLRLIYYSITENDLQKG
48 - 75	YSITENDLQKGDLSEGYDASREKKSSFS
58 - 75	GDLSEGYDASREKKSSFS

a) The peptides were synthesized according to the Merrifield solid-phase technique (20).
b) Sequences of peptides were from Patten *et al.* (31).

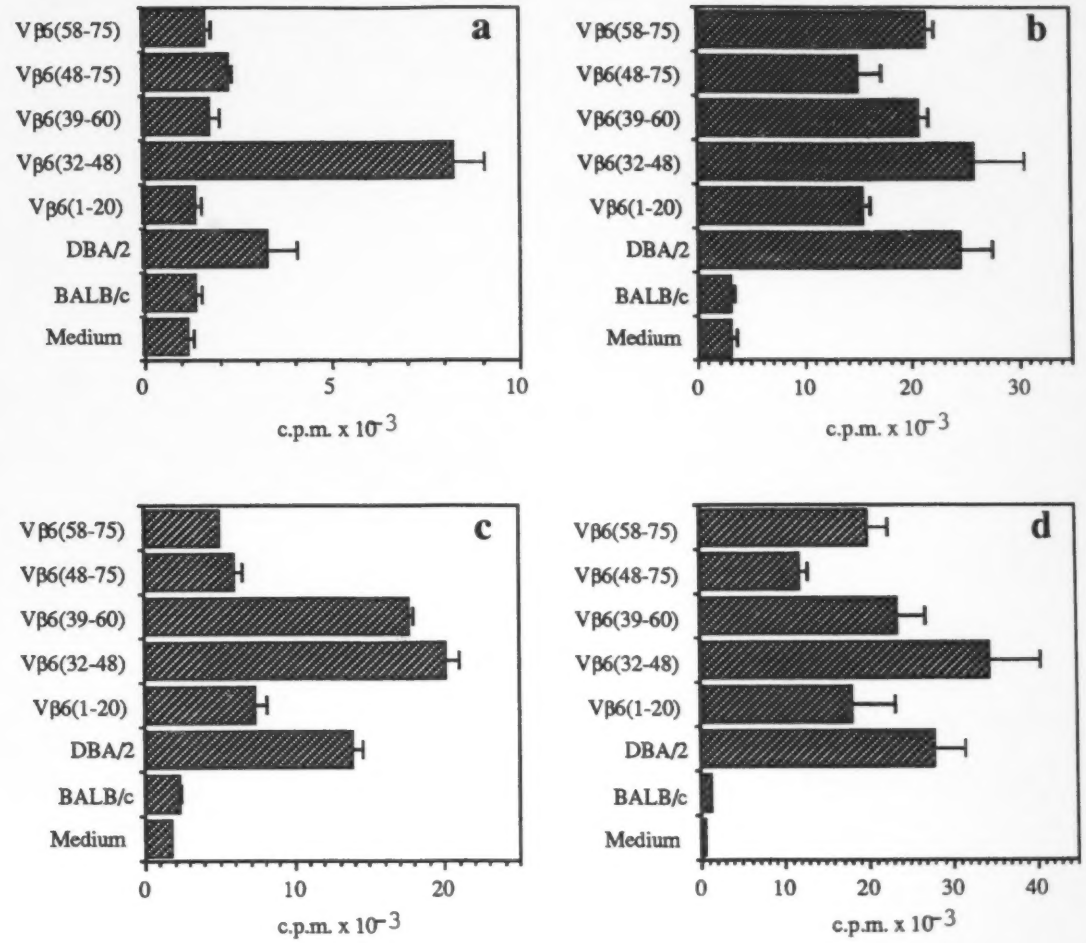

Figure 2.1. Inhibition of recognition of Mls-1^a by Vβ6$^+$ T cell hybridomas with Vβ6 peptides. Peptides were added at a final concentration of 75 μM to microcultures of 1 x 10^5 T cell hybridomas and 1 x 10^6 DBA/2J irradiated (3000 rads) spleen cells, and IL-2 production was determined as described in Material and Methods with CTL-L cells. The T cell hybridomas used are as follows: (a) B11; (b) B4; (c) B2; (d) B5.

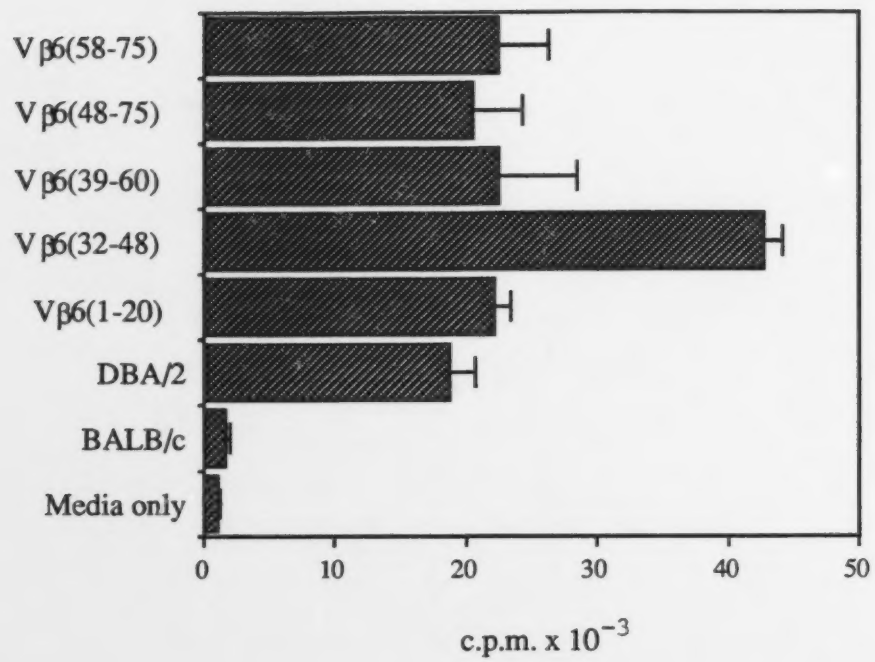

Figure 2.2 Vβ6 peptides cannot inhibit recognition of Mls-1^a by the $V\beta8^+$ T cell hybridoma, B9. Different Vβ6 peptides were added at a final concentration of 75 μM to microcultures of 1 x 10^5 T cell hybridoma and 0.5 x10^6 DBA/2 irradiated (3000 rads) spleen cells, and IL-2 production was determined as in Fig. 2.1.

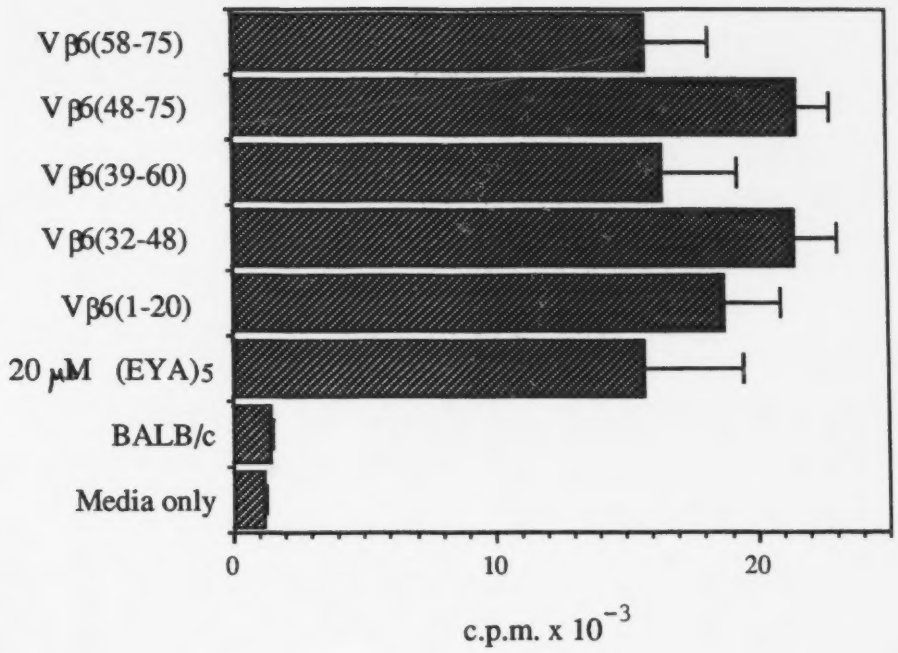

Figure 2.3. Vβ6 peptides cannot inhibit recognition of peptide antigen, $(EYA)_5$ at 20 μM, by the $V\beta6^+$ T cell hybridoma, B11. Different Vβ6 peptides were added at a final concentration of 75 μM to microcultures of 1 x 10^5 T cell hybridoma and 1 x 10^6 BALB/c irradiated (3000 rads) spleen cells in the presence of 20 μM $(EYA)_5$, and IL-2 production was determined as in Fig. 2.1.

Bibliography

1. Ziegler, H.K., E.R. Unanue. 1982. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells Proc. Natn. Acad Sci U.S.A. 79:175-178.

2. Schwartz, R.H. 1985. T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Ann. Rev. Immunol. 3:237-261.

3. Kronenberg, M., G. Sui, L.E. Hood, and N. Shastri. 1986. The molecular genetics of the T cell antigen receptor and T cell antigen recognition. Ann. Rev. Immunol. 4:529-591.

4. Raulet, D.H. 989. The structure, function, and molecular genetics of the γ/δ T cell receptor. Ann. Rev. Immunol. 7:175-207.

5. Demic, Z., H. von Boehmer, and M. Steinmetz. 1986. The role of T-cell receptor α and β genes in MHC-restricted antigen recognition. Immunol. Today 10:308-311.

6. Winoto, A., J.L. Urban, N.C. Lan, J. Goverman, L. Hood, and D. Hansburg. 1986. Predominant use of a Vα gene segment in mouse T-cell receptors for cytochrome c. Nature 324:679-681.

7. Sorger, S.B., S.M. Hedrick, P.J. Fink, M.A. Bookman, and L.A. Matis. 1987. Generation of diversity in t cell receptor repertoire specific for pigeon cytochrome c. J. Exp. Med. 165:279-301.

8. Urban, J.L., V. Kumar, D.H. Kono, C. Gomez, S.J. Horvath, J. Clayton, D.G. Ando, E.E Sercarz, and L. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell 54:577-592.

9. Acha-Orbea, H, D.J. Mitchell, L. Timmermann, D.C. Wraith, G.S. Tausch, M.K. Waldor, S.S. Zamvil, H.O. McDevitt, and L.

Steinman. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis anlows specific immune intervention. Cell 54:263-273.

10. Zamvil, S.S., D.J. Mitchell, N.E. Lee, A.C. Moore, M.K. Waldor, K. Sadai, J.B. Rothbard, H.O. McDevitt, L. Steinman, and H. Acha-Orbea. 1988. Predominant expression of a T cell receptor V gene subfamily in autoimmune encephalomyelitis. J. Exp. Med. 167:1586-1596.

11. Frankel, W.N., C. Rudy, J.M. Coffin, and B.R. Huber.1991. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. Nature 349:526-528.

12. Abe, R., M.S. Vacchio, B. Fox, and R.J. Hodes. 1988. Preferential expression of the T-cell receptor Vβ3 gene by Mls^c reactive T cells. Nature 335:827-828.

13. MacDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha-Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. Nature 332:40-45.

14. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature 332:35-40.

15. Happ, M.P., D.L. Woodland, and E. Palmer. 1989. A third T-cell receptor β-chain variable region gene encodes reactivity to Mls-1^a gene products. Proc. Natn. Acad Sci U.S.A. 86:6293-6296.

16. Lynch, D.H., R.E. Gress, B.W. Needleman, S.A. Rosenberg, and R.J. Hodes. 1985. T cell responses to Mls determinants are restricted by cross-reactive MHC determinants. J. Immunol. 134:2071-2078.

17 Novak, Z., M. Boyer, P. Kilgannon, E. Fraga, A. Fotedar, and B. Singh. 1992. Unusually diverse T cell response to a repeating tripeptide epitope. Cell. Immunol. 140:206-218.

18. Boyer, M., Z. Novak, A. Fotedar, and B. Singh. 1988. Contribution of antigen processing to the recognition of a synthetic peptide antigen by specific T cell hybridomas. J. Mol. Rec. 1:99-106.

19. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of the T cell αβ receptor. EMBO J. 7:3745-3755.

20 Fotedar, A., M. Boyer, W. Smart, J. Widtman, E. Fraga, and B. Singh. 1985. Fine specificity of antigen recognition by T cell hybridoma clones specific for Poly-18: a synthetic polypeptide antigen of defined sequence and conformation. J. Immunol. 135:3028-3033.

21. Janeway, C.A. Jr., J. Yagi, P.J. Conrad, M.E. Katz, B. Jones, S. Vroegop, and S. Buxser. 1989. T-cell responses to Mls and to bacterial proteins that mimic its behavior. Immunol. Rev. 107:61-88.

22. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T-cell-receptor β-chain that interact with *S. aureus* toxin superantigens. Nature 346:471-473.

23. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor β chain that interacts with the self-superantigen Mls-1^a. Cell 61:1365-1374.

24. Pullen, A.M., J. Bill, R.T. Kubo, P. Marrack, and J.W. Kappler. 1991. Analysis of the interaction site for the self superantigen Mls-1^a on T cell receptor Vβ. J. Exp. Med. 173:1183-1192.

25. Hengartner, H., B. Odermatt, R. Schneider, M. Schreyer, G. Walle, H.R. MacDonald, and R.M. Zinkernagel. 1988. Deletion

of self-reactive T cells before entry into the thymus medulla. Nature 336:388-390.

26. White, J., A. Herman, A.M. Pullen, R. Kubo, J.W. Kappler, and P. Marrack. 1989. The Vβ-specific superantigen staphylococcal enterotoxin B: Stimulation of mature T cells and clonal deletion in neonatal mice. Cell 56:27-35.

27. Inaba, M., K. Inaba, M. Hosono, T. Kumamoto, T. Ishida, S. Muramatsu, T. Masuda, and S. Ikehara. 1991. Distinct mechanisms of neonatal tolerance induced by dendritic cells and thymic B cells. J. Exp. Med. 173:549-559.

28. Vandenbark, A.A., G. Hashim, H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541-544..

29. Howell, M.D., S.T. Winters, T. Olee, H.C. Powell, D.J. Carlo, S.W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. Science 246:668-670.

30. Offner, H., G.H. Hashim, A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. Science 251:430-432.

31. Patten, P., T. Yokota, J. Rothbard, Y.-H. Chien, K.-I. Arai, M.M. Davis. 1984. Structure, expression and divergence of T-cell receptor β-chain variable regions. Nature 312: 40-46.

CHAPTER III

Characterization of murine T cell response to peptides of self T cell receptor β chains[1]

Introduction

The TCR for Ag is composed of an α and β or γ and δ disulfide heterodimer co-expressed with a complex of five polypeptides termed CD3. Each of the α, β, γ and δ chains contains a variable N-terminal portion which interacts with peptide antigen presented by MHC molecules (1, 2). Although the T cell response to antigen is heterologous in terms of Vα and Vβ usage, there are several examples of dominant Vα or Vβ usage in cellular immune responses to certain peptide antigens (3) and to superantigens including staphylococcal enterotoxins (4) and Mls^a (5). Dominant T cell receptor usage in an immune response suggests the possibility of clonal specific regulation of that response. It has been shown that immunization with a peptide epitope of the Vβ8 TCR can protect Lewis rats from induction of EAE (6, 7). However, there has been recent evidence that such immunization does not lead to protection in every case (8). Little is known about immune responses to determinants of the TCR, especially in mice, therefore

---

1 A version of this chapter has been submitted for publication. MacNeil, Fraga, and Singh. 1992.

we have characterized the murine immune response to several TCR determinants in the context of immunogenicity and self tolerance. Tolerance results from the elimination or inactivation of self-reactive cells (9). Much of the experimental work on tolerance by clonal deletion has been done with the Mls loci (10, 11), which have recently been identified as products of different endogenous mouse mammary tumour viruses (12). Mouse strains of the Mls-1^a haplotype carry the *mtv*-7 and delete V$\beta6^+$, V$\beta7^+$, V$\beta8.1^+$ and V$\beta9^+$ T cells (13 -15) and mice strains of the Mls-2^a haplotype carry *mtv*-13 and delete V$\beta3^+$ T cells (16). We were interested in using Mls-1^a and Mls-1^b mice strains which delete or do not delete, respectively, T cells bearing various Vβ TCR to characterize the response to self peptides of the Vβ region of the TCR. We speculated that Mls-1^a mice would respond to peptides of V$\beta6$ and that Mls-1^b mice, which have V$\beta6^+$ peripheral T cells, would not respond to the same peptides. We tested a panel of five peptides spanning most of the variable V$\beta6$ sequence for T cell responses in BALB/c, DBA/2J, CBA/CaJ, CBA/J, C57Bl/6J and D1.LP mice and the amino terminal peptide of other Vβs in BALB/c and DBA/2J mice. Not all of the peptides tested were immunogenic, however, priming with some of the peptides in CFA elicited T cell responses in some mice strains independently of their "deletor status". Response of the mice strains correlated with H-2 haplotype, thus the response to these self peptides may exhibit an Ir effect. We show that T cell and B cell responses to determinants of the V$\beta6$ region of the TCR can be generated after immunization with peptides of the TCR in

several strains of mice, however, the B cell response is limited to the peptides of the TCR and does not extend to the intact TCR molecule. Our results indicate that T cells specific for self peptides are not deleted in the thymus nor inactivated in the periphery in spite of a potential reactivity to self. Immunoregulatory properties of these cells remain to be elucidated particularly in light of the conflicting results reported in the rat EAE model (6 - 8).

Materials and methods

Mice

DBA/2J, CBA/CaJ, CBA/J, C57Bl/6J, and D1.LP mice were purchased from the Jackson Laboratory, Bar Harbor, ME. BALB/cCr mice were bred at the Ellerslie Animal Farm of the University of Alberta.

Peptides

Peptides were prepared by the Merrifield solid-phase technique on a Beckman 990C Peptide Synthesizer (Palo Alto, CA), as previously described (17). The crude preparations were purified by HPLC on a C-18 reverse phase semipreparative SynChropak RP-P column (Synchrom, Linden, IN), and peptides were eluted with a linear gradient from water to acetonitrile (1.37% acetonitrile/min). The composition of the peptides was confirmed by amino acid analyses using a Beckman System 6300 amino acid analyzer. All peptides gave acceptable ratios of the various amino acids. For functional assays, peptides were dissolved in saline by adjusting pH to 7.2 with 0.1 N NaOH and were sterilized by filtration through a 0.22 μm filter.

Immunizations and response to Ag

For T cell response, mice were immunized in the footpads with 50 μg of peptide emulsified with CFA (Sigma, St. Louis, MO). Eight days later, popliteal lymph nodes were removed and cells were purified over nylon wool columns (18). Purified cells were cultured in 96-well plates (Flow, McLean, VA) at 4 x 10^5 or 2 x 10^5 cells/well with

syngeneic irradiated (3000 rad) spleen cells as APC at 1 x 10^6 cells/well in the presence or absence of peptide in 200 µL of culture medium (RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FCS (Bocknek, Canada), 10 mM Hepes, 2 mM L-glutamine, $5x10^{-5}$ M 2-ME, and 1 U/ml penicillin-streptomycin). Concentrations of peptides tested were 250 µg/mL, 25 µg/mL, and 2.5 µg/mL; PPD (20 µg/mL) served as a positive control for T cell proliferation. After four days of culture at 37°C, and 7% CO_2, cultures were pulsed with 1 µCi of [^{3}H]dThd (NEN Dupont, Boston, MA) for 16-20 h. Incorporation of [^{3}H]dThd was measured using a liquid scintillation counter (LKB Instruments, Gaithersburg, MD).

For B cell response, mice were immunized in one footpad with 50 µg of peptide emulsified with CFA and, ten days later, immunized again in the opposite footpad with 50 µg of peptide emulsified with IFA (Sigma, St. Louis, MO). Twenty-five days later, mice were bled from the tail vein, and sera were tested for antibody (Ab) titre by ELISA (19). Plates were coated overnight with 10 µg/mL of peptide. Normal mouse serum (NMS) was used as a negative control, goat-anti-mouse IgG-alkaline phoshatase conjugate as the indicator antibody, and p-nitrophenyl phosphate (Sigma) as a substrate in diethanolamine buffer (pH 9.6). Optical density (O.D.) of the p-nitrophenol product was measured at 405 nm by a kinetic microplate reader (Molecular Devices, Menlo Park, CA).

Cytokine assays

For the determination of cytokine production, purified popliteal lymph nodes from immunized mice were prepared and cultured as described above in 250 µl of culture medium, in the presence or absence of 50 µg/ml of the immunizing peptide. After 48 h, supernatants were collected and assayed for IL-2 and IL-4 content in secondary cultures of the IL-2/IL-4 dependent HT-2 cell line. Cells were cultured in 50% or 25% of test supernatant in the presence or absence of anti-IL-2 MAb or anti-IL-4 MAb for 24 h, then incorporation of [^{3}H]dThd was measured as above. Supernatants of cultured T cells were also tested for the production of IFNγ by the Intertest mouse IFNγ ELISA Test Kit (Genzyme, Cambridge, MA).

MAb

The mAb MK-D6 (anti-I-A^d), 10-3.6.2 (anti-I-A^k), 17-3-5S (anti-I-E^k), 16-3-1N (anti-K^k), 15-5-5S (anti-D^k), and 34-5-8S (anti-D^d) were used in blocking experiments as ascites fluid as described before (20). GK1.5 (anti-CD4) and 2.43 (anti-CD8) were precipitated with 40% (v/v) saturated ammonium sulfate from supernatants of cell cultures and subsequently dissolved in PBS. Both ascites fluid and precipitated mAb preparations were extensively dialyzed against PBS before use. Hybridoma cells producing these mAb were purchased from American Type Culture Collection, Rockville, MD. 44.22.1 (anti-Vβ6), KJ16 (anti-Vβ8.1,2), M7/20 (anti-IL-2 receptor) and S4B6 (anti-IL-2) were used as

supernatants of cell cultures. Hybridoma cells 44.22.1 were provided by H. Hengartner (Zurich, Switzerland), KJ16 by J.W. Kappler (Denver, CO), M7/20 by T.B. Strom (Boston, MA), and S4B6 by T.R. Mosmann (Edmonton, Canada). The anti-IL-4 mAb was purchased from Genzyme (Cambridge, MA).

Hybridomas

The T cell hybridoma A.1.1 is poly-18-specific and I-A^d restricted and has been previously described (20). A.1.1 is also reactive to a synthetic peptide K4 when presented by normal or fixed APC (21) and is Vβ6 positive (Kilgannon, et. al., submitted). The B cell hybridoma TA3 expresses I-$A^{d/k}$/I-$E^{d/k}$ (22) and was used in competitive inhibition assays as APC. The IL-2 dependent cell line CTL-L was used to assay IL-2 and IL-4 content in the supernatant of A.1.1 cells upon activation with Ag as described before (26).

Ab blocking of T cell proliferation assay

In blocking studies, 4 x 10^5 responding cells were cultured (as described for the T cell proliferation assay) with 25 μg/mL of peptide. Ascites fluid and concentrated supernatants of mAbs were added directly to cultures for the entire five-day period at a final concentration of 80 μg/mL total protein. Experimental values were calculated as percent inhibition of control incorporation by the following formula:

$$\frac{\text{c.p.m. without mAb - c.p.m. with mAb}}{\text{c.p.m. without mAb}} \times 100\%$$

Competitive inhibition assay

A.1.1 cells (1 x 10^5 cells/well) were cultured with glutaraldehyde fixed TA3 cells (2 x 10^5 cells/well) and 20 μg/mL of K4, in the presence or absence of Vβ6 peptides (400 μg/mL, 40 μg/mL, 4 μg/mL). After 24 h supernatants were collected and assayed for IL-2 content in secondary cultures of CTL-L cells. Cells were cultured in 50% or 25% of test supernatants for 40 h, then pulsed with 1 μCi of [^{3}H]dThd (NEN Dupont, Boston, MA) for 24 h. Incorporation of [^{3}H]dThd was measured using a liquid scintillation counter (LKB Instruments, Gaithersburg, MD). Experimental values were calculated as percent inhibition of control incorporation as in the mAb blocking assay:

$$\frac{\text{c.p.m. without peptide - c.p.m. with peptide}}{\text{c.p.m. without peptide}} \times 100\%$$

Indirect immunofluorescence

Murine peripheral blood lympocytes (PBL) were purified from whole blood over Lympholite M (Cedarlane, Canada). A.1.1 or PBL or purified lymph node cells were washed in PBS (pH 7.4) containing 2% FBS and 0.05% NaN_3, then incubated on ice for 1 h with 100 μL of 1:10 mouse anti-sera or 44.22.1 culture supernatants. NMS and KJ16 served as negative controls for mouse anti-sera and 44.22.1, respectively. Cells were washed once in cold

PBS and incubated on ice for 1 h with 100 μL of goat-anti-mouse FITC conjugate (1:50) or goat-anti-rat FITC conjugate (1:100) (Tago, Burlingame, CA). Cells were washed again and resuspended in PBS containing 2% formalin. Samples were analyzed with a Becton Dickinson FACScan (Mountain View, CA).

RESULTS

T cell responses to self Vβ peptides

To test the immunogenicity of self Vβ peptides, mice were primed in the hind footpads with various Vβ peptides emulsified in CFA. Eight days later, T cells from draining lymph nodes were purified over nylon wool columns and cultured with the peptides and irradiated syngeneic spleen cells as APC. Five peptides of Vβ6 were tested, Vβ6(1-20), Vβ6(32-48), Vβ6(39-60), Vβ6(48-75), Vβ6(58-75), as well as one peptide of Vβ8.1(1-20). BALB/c, CBA/CaJ, and C57Bl/6J do not delete $V\beta6^+$ or $V\beta8.1^+$ T cells. Therefore the responses to these peptides were compared between strains of matching MHC haplotype and different Mls phenotypes: BALB/c and DBA/2, CBA/CaJ and CBA/J, C57Bl/6J and D1.LP. Vβ3(1-20) served as a negative control since BALB/c mice delete $V\beta3^+$ T cells, as do DBA/2 mice. Vβ8.3 served as a positive control since DBA/2J and BALB/c mice do not delete $V\beta8.3^+$ T cells. Also, within the selection of Vβ peptides, the non-immunogenic peptides serve as internal controls for the specificity of response (see Table 3.1 for peptide sequences). BALB/c mice respond weakly to Vβ6(1-20) and Vβ6(39-60), and strongly to the remaining peptides tested with the exception of Vβ8.1(1-20) (Table 3.2). The response pattern of DBA/2 mice to Vβ-peptides is similar to that of BALB/c mice except that DBA/2 mice also do not respond Vβ3(1-20), or Vβ8.3(1-20). CBA/CaJ and CBA/J mice respond weakly to Vβ6(39-60), and strongly to Vβ6(48-75), Vβ6(58-75) and Vβ3(1-20), but not to Vβ6(1-20) or Vβ6(32-48). C57Bl/6J mice respond weakly to

Vβ6(58-75) and perhaps Vβ6(39-60), but not to the other Vβ6-peptides tested. D1.LP mice do not respond to Vβ6(1-20) or Vβ6(58-75). Thus, responsiveness to some of the peptides appears to be determined by H-2 haplotype. The H-2^d strains respond to Vβ6(1-20) and Vβ6(32-48); the H-2^d and H-2^k strains respond to Vβ6(48-75) and Vβ6(58-75); the H-2^b strains respond weakly or not at all to any of the tested Vβ6 peptides.

Ab response to Vβ6 peptides

To determine if lack of tolerance to the Vβ6 peptides is also present in the B cell response, Vβ6(1-20), Vβ6(32-48), Vβ6(39-60), and Vβ6(48-75) were tested in BALB/c mice for induction of Ab. After one immunization and one boost, Ab titres in three groups of mice are evident (Fig 3.1). Vβ6(1-20) induced higher titres than Vβ6(39-60) and Vβ6(48-75) in BALB/c mice, and Vβ6(32-48) did not induce any Ab. Vβ6(1-20) was also tested for Ab induction in DBA/2J mice with results similar to BALB/c (data not shown). To test if the anti-peptide sera had any affinity for the intact Vβ6 TCR on the cell surface, antisera were used to stain a Vβ6$^+$ T cell hybridoma in indirect immunofluorescence. Fluorescence-flow cytometry analysis shows that the anti-sera from Vβ6(1-20) or Vβ6(48-75) primed mice (1:10 dilution) has no affinity for Vβ6$^+$ T cell hybridoma, A.1.1, however, staining with 44.22.1 (anti-Vβ6 TCR) shows the expression of TCR by the hybridoma (Fig. 3.2a). Thus, the anti-peptide antibodies recognize a fragment of Vβ6 TCR, but do not recognize the intact Vβ6 TCR. To further test that the

anti-TCR peptide response does not extend to the TCR, we screened the BALB/c mice which had the highest Ab titres for Vβ6(1-20) for the presence of Vβ6$^+$ PBL. We observed that Vβ6$^+$ PBL were present in normal frequency (8.0%) in these mice after forty days following immunization with Vβ6(1-20) compared to untreated BALB/c Vβ6$^+$ PBL (7.3%) (Fig. 3.2b).

Cytokine production by T cells responding to peptides of Vβ6 TCR. It is interesting that Vβ6(32-48) induces a strong T cell response as measured by the *in vitro* T cell proliferation assay, but no Ab response, and that Vβ6(39-60) induces little or no T cell response, but a strong Ab response. Therefore we tested the production of IL-2, IL-4, and IFNγ by T cells stimulated with these peptides to determine if Vβ6(32-48) induces the Th1 subset of T cells and Vβ6(39-60) induces the Th2 subset of T cells, as implied by the above results. Fig. 3.3a shows that IL-2 and/or IL-4 are produced by T cells primed and cultured with Vβ6(32-48), but not with Vβ6(39-60). The lymphokine produced by the former is IL-2, as demonstrated by the blocking with anti-IL-2 MAb but not anti-IL-4 MAb (Fig. 3.3b). The T cells stimulated with Vβ6(32-48) also produce IFNγ, consistent with the cytokine profile of Th1 cells (Fig. 3.4).

Vβ6 usage among T cells responding to peptides of Vβ6 TCR
To determine if the cellular response to Vβ6 peptides affects the frequency of Vβ6 among responding T cells, the frequency of Vβ6$^+$

cells was measured after stimulation with Vβ6 peptides *in vitro*. BALB/c mice were immunized with either saline, Vβ6(1-20), or Vβ6(48-75) emulsified in CFA and the draining lymph nodes were prepared as described in the Materials and Methods for the proliferation assay. The cells were then stimulated *in vitro* with either the Vβ6 peptide or purified protein derivative (PPD, Statens Seruminstitut, Copenhagen, Denmark) as a negative control at 25 μg/mL for 4 days. Viable cells were prepared for flow cytometry. In Table 3.3, are listed the frequencies of $V\beta6^+$, $V\beta8^+$, and $CD4^+$ cells after 4 days culture. It is presumed that during this *in vitro* stimulation, the majority of viable cells are those responding to the antigen in culture. The frequencies of Vβ6 and Vβ8 among the responding cells are similar for all groups. Regardless of *in vivo* priming with Vβ6 peptides and/or *in vitro* stimulation with Vβ6 peptides, the frequencies of $V\beta6^+$ and the control $V\beta8^+$ T cells are approximately 10% and 15%, respectively. Thus, although Vβ6 is present in the repertoire of T cells responding to Vβ6 peptides, the repertoire is not skewed in the frequency of $V\beta6^+$ T cells.

Anti-class II mAb blocks in vitro proliferation of primed T cells

To determine the MHC molecules which present Vβ6 peptides, primed T cells were stimulated *in vitro* with peptide and syngeneic irradiated spleen cells as APC in the presence of various mAb (Fig. 3.5). Since many of the peptides tested in BALB/c and DBA/2J mice are from the amino terminal peptide of various Vβ genes, Vβ6(1-20) was chosen for this experiment in BALB/c mice. The response

to Vβ6(1-20) was blocked by MK-D6 (anti-I-A^d) but not by the isotype-matched control mAb, 10-3.6.2 (anti-I-A^k) (Fig. 3.5a). The response was also blocked to a lesser extent by GK1.5 (anti-CD4), but not by 35-5-8S (anti-D^d) or 2.43 (anti-CD8) antibodies. Comparable results were obtained in blocking experiments with CBA/CaJ mice responding to Vβ6(48-75); Vβ6(1-20) could not be used in this experiment since CBA/CaJ mice do not respond to Vβ6(1-20) (Fig. 3.5b). Both 10-6.3.2 (anti-I-A^k) and 17-3-5S (anti-I-E^k) blocked primed CBA/CaJ T cell proliferation in response to Vβ6(48-75). Proliferation was slightly inhibited by 16-3-1N (anti-K^k) but not by 2.43 (anti-CD8) antibodies. None of the mAb were toxic to the purified T cells at the concentration used in the blocking assay, as determined by trypan blue exclusion (data not shown). Thus the T cell response to Vβ6 peptides requires presentation by class II MHC molecules. The response is partially blocked by anti-CD4 mAb, indicating that CD4-bearing T cells are involved in recognition of Vβ6 peptides.

Vβ6 peptides inhibit presentation of Ag to the T cell hybridoma, A.1.1

Competitive inhibition experiments were performed with the I-A^d restricted T cell hybridoma, A.1.1, and fixed TA3. A.1.1, which responds to the peptide, K4, in the presence of I-A^d, was cultured with glutaraldehyde fixed TA3 and K4 in the presence of various concentrations of Vβ6 peptides. The use of fixed TA3 abrogated possible contributions to peptide binding to I-A due to processing

by APC. At lower concentrations, all six Vβ6 peptides inhibited the presentation of K4 antigen to A1.1 T cell hybridoma (Fig. 3.6). At higher concentrations of competing peptide, inhibition was similar to that at lower concentrations. None of the peptides were toxic to the T cell hybridoma at any concentration tested as determined by trypan blue exclusion (data not shown). Vβ6(39-60) was the strongest inhibitor, even though Vβ6(39-60) was the least immunogenic of all the Vβ6 peptides according to the T cell proliferation assay. Since none of the peptides were toxic to the responding A.1.1, the Vβ6 peptides inhibit Ag presentation by competing for binding to I-A.

Discussion

Our demonstration of T cell and B cell responses to self peptides of the TCR indicate the absence of tolerance to some self epitopes of the TCR in several strains of mice. The B cell response was peptide-specific and the peptide-specific antisera did not react with the intact TCR on cells. The response to these peptides at the T cell level is interesting.

There have been a few reports of T cell recognition of self antigens, including myelin basic protein (23), β_2-microglobulin, haemoglobulin (24), and I-A (25, 26). Our results show that the lack of tolerance extends to the TCR. The response patterns of the different strains of mice are not determined by the Mls haplotype, ie. whether a particular Vβ is deleted or not. BALB/c and DBA/2J mice both respond to most of the Vβ6-peptides tested even though DBA/2J mice delete $V\beta6^+$ T cells and BALB/c mice do not. BALB/c mice respond to Vβ3(1-20) and DBA/2J mice do not, even though both delete $V\beta3^+$ T cells and are of the same haplotype ($H\text{-}2^d$). Neither BALB/c nor DBA/2J mice respond to Vβ8.1(1-20) and both respond to Vβ8.3(1-20), when DBA/2J mice delete $V\beta8.1^+$ T cells and neither BALB/c nor DBA/2J mice delete $V\beta8.3^+$ T cells. CBA/CaJ and CBA/J mice both respond to the same three peptides of the six Vβ6 peptides tested, even though CBA/J mice delete $V\beta6^+$ and $V\beta3^+$ T cells and CBA/CaJ mice do not. Therefore, no differences in response patterns can be attributed to the $Mls\text{-}1^a$ phenotype. However, the pattern of response to the different peptides

correlates to H-2 haplotype. The H-2^d strains (BALB/c and DBA/2J) respond to Vβ6(1-20), Vβ6(32-48), Vβ6(48-75) and Vβ6(58-75), whereas the H-2^k strains (CBA/CaJ and CBA/J) respond to Vβ6(48-75) and Vβ6(58-75). The H-2^b strains respond only weakly to Vβ6(58-75) in the case of C57Bl/6J, or not at all in the case of D1.LP mice. Thus deletion of Vβ6$^+$ T cells does not influence tolerance or responsiveness to the peptides of Vβ6. We expected that Mls-1^a mice which delete Vβ6$^+$ T cells would respond to the Vβ6 peptides and that Mls-1^b mice which do not delete Vβ6$^+$ T cells would not respond to the peptides. This was not the case. Mice which express Vβ6 on a portion of their peripheral T cells can respond to some peptide fragments of Vβ6, even though this molecule is "self". This may be explained by the fact that Vβ6$^+$ cells are present in the thymus medulla of newborn mice for up to 7 days following birth (27) and remain in the thymus cortex in the adult mice of Mls-1^a (deletor) strains (28). Since the injection of antigen within one day after birth can render mice tolerant to that antigen (29), the presence of Vβ6$^+$ cells during the time when "self" is "learned" may prevent any differences in tolerance between Mls-1^b and Mls-1^a mice. Vβ-peptides are immunogenic in both deletors and non-deletors, however, indicating a lack of tolerance to some epitopes of an autoantigen.

It is interesting to note that the six strains tested respond weakly to Vβ6(39-60), however, this region of the Vβ8 TCR is reported to be immunogenic in rats (6). Whether low response is due to the

different Vβ gene segment or the different species can not be determined. Poor immunogenicity of Vβ6(39-60) in mice implies that the regulation of EAE with this region of Vβ8 in rats (6, 7) may not be a general phenomenon which can be applied across species in similar autoimmune diseases. Even when a particular TCR is implicated in the pathogenesis of an autoimmune disease or any immune response, the regulatory region of the TCR must be identified. This may be due to differences in antigen processing between the different TCR molecules, which would generate TCR-peptides from different regions of the TCR. The peptides having a regulatory effect would be those which are processed and presented naturally by either T cells or APC.

B cell responses to three of the Vβ6 peptides are also evident by Ab titres in the sera of immunized mice. Ab titres to Vβ6(1-20), Vβ6(39-60) and Vβ6(48-75), but not to Vβ6(32-48) could be detected in BALB/c mice. Our results suggest that Vβ6(39-60) could induce detectable Ab titres even though the T cell response to this peptide is very low. This minimal T cell response must be sufficient to provide the observed help to B cells producing anti-Vβ6(39-60) Ab. Conversely, Ab titres to Vβ6(32-48) could not be detected by the ELISA technique, in spite of a strong response in the *in vitro* T cell proliferation assay. Possibly, the T cell subset induced by this peptide is unable to provide help to B cells or B cells producing anti-Vβ6(32-48) Ab are deleted in BALB/c mice.

The above results prompted us to investigate this system further. We tested the cytokines produced by T cells primed and stimulated *in vitro* with Vβ6(32-48) or Vβ6(39-60). Consistent with Vβ6(32-48) inducing a Th1 subset, T cells stimulated with this peptide produced detectable levels of IL-2 and IFNγ. T cells stimulated with Vβ6(39-60), did not produce IL-2 or IFNγ, but neither was IL-4 detected in the supernatants of these T cells. This is likely due to the insensitivity of the bioassay for IL-4. Concentrations of IL-4 in supernatants below 4 ng/mL were not detectable in this assay; this would be insufficient to detect many Th2 type clones (30). The induction of Th1 type T cells by Vβ6(32-48) would account for the results from the T cell proliferation and Ab production assays. It has been observed that different antigens can preferentially induce one T cell subset over the other (31, 32). Even different epitopes of the same protein can induce different types of immune responses (33, 34). Vβ6(32-48) and Vβ6(39-60) are further examples of epitopes preferentially inducing different subsets of T cells.

There are evidently B cells with specificity for some peptide fragments of the Vβ6 TCR. This Ab specificity however does not extend to the intact TCR on the surface of T cells (Fig. 3.1a). Antisera from BALB/c and DBA/2J mice which can bind the peptide fragment Vβ6(1-20) cannot bind to the Vβ6$^+$ T cell hybridoma, A.1.1. This result is in contrast to previous findings in which rabbit anti-sera generated against the VDJ region on the β chain of the TCR expressed by an encephalitogenic T cell line (35) or rat anti-sera

generated against Vβ8(39-59) (36) were able to bind to Vβ8$^+$ encephalitogenic T cells. Anti-peptide Abs may not recognize intact TCRs because (1) the peptide sequence may not be on the surface of the TCR and so be inaccessible to the Ab, or (2) the structure of the peptide recognized by the Ab differs from the structure of the peptide sequence in the intact TCR. In addition, since the anti-sera were generated in mice expressing the Vβ6 TCR, any B cells producing Abs able to bind to the intact TCR could be either anergized or deleted in these mice (37). Thus, anti-Vβ6 TCR Abs were not generated. In addition, anti-Vβ6(1-20) Abs present in the sera do not lead to depletion of Vβ6$^+$ T lymphocytes in the periphery (Fig. 3.1b). Therefore, the anti-Vβ6 peptide response by B cells does not lead to an anti-receptor response.

The T cell response to peptides of the TCR is primarily by class II-restricted, CD4$^+$ T cells as shown by mAb blocking *in vitro* (Fig. 3.5). We tested the response to Vβ6(1-20) in BALB/c mice. Anti-I-A^d mAb (MK-D6) and to lesser extent anti-CD4 mAb (GK1.5) blocked proliferation of T cells in response to Vβ6(1-20). Therefore class II MHC molecules are required for the presentation of Vβ6 peptides to primed T cells. It is evident that all of the five Vβ6-peptides tested can competitively inhibit presentation of K4 by I-A^d (Fig. 3.6). The Vβ6-peptides also have sequences similar to putative I-A^d binding motifs (38), suggesting that the Vβ6-peptides may inhibit presentation of K4 by competitively binding the I-A^d molecule. It

has been previously shown that self-peptides can bind MHC molecules (24 - 26, 39, 40).

We also examined the usage of Vβ6 by T cells responding to two of the Vβ6 peptides to determine if the cellular response to regions of a particular Vβ can skew the repertoire of responding T cells. T cells responding to Vβ6(1-20) or Vβ6(48-75) were selected by immunization and subsequent *in vitro* stimulation with the respective Vβ6 peptides. CFA-primed and PPD-stimulated T cells served as a negative control and the frequencies of $V\beta6^+$ T cells were compared among the groups. The frequencies of $V\beta6^+$ T cells in all groups were within the normal range of frequencies of $V\beta6^+$ T cells in lymph nodes of BALB/c mice from 7% (28) to 11% (5). The control, Vβ8, was also expressed within the normal range from 15% to 20% of peripheral lymph node cells (41). Thus the repertoire of T cells responding to Vβ6 peptides does not have a greater or lesser proportion of $V\beta6^+$ T cells than normal.

These results have several implications. First, T cells reactive with some self-peptides are not deleted in the thymus and are able to respond to antigen in the context of MHC. This escape from thymic deletion cannot be attributed to the absence of the self molecules in the thymus. It is possible that peptide fragments of TCR are present in the thymic environment since the majority of T cells in the thymus are deleted and presumably degraded and presented by resident phagocytic cells. The peptide fragments present in the

thymic environment may not be the same as the peptides tested in this study, thus T cells may escape deletion because, as has been suggested (42), tolerance to all self epitopes may not be necessary, since not all self epitopes may be generated from a primary sequence *in vivo*. Alternatively T cells may escape deletion because of low affinity TCRs specific for the naturally presented self epitopes.

Second, assuming that positive selection is necessary for T cells to exit the thymus, the presence of these self peptide-reactive T cells in the periphery implies that these cells were positively selected. The nature of positive selection is unclear, but it is generally perceived as a low-affinity interaction between the TCR and self-peptide associated with MHC (43). It has been speculated (24) that self-peptides may be presented on thymic epithelium and mimic the universe of foreign peptides and there is some evidence that self peptides can influence the T cell repertoire (44, 45). The TCR is a possible candidate as a source of a highly diverse set of peptides. The presence of TCR peptide reactive T cells is consistent with a role of peptides of the TCR in positive selection of the T cell repertoire.

Finally, T cell responses which are dominated by usage of certain TCR genes may be specifically regulated through anti-TCR-peptide T cells. Anti-Vβ8(39-59) T cells have been shown to prevent the induction of experimental autoimmune encephalomyelitis by $V\beta8^+$

autoimmune T cells in rats (6), although this finding has not been supported by others (8, 46). The mechanism of regulation through immunization with peptides of the TCR is unclear. We have previously shown that some of the Vβ6 peptides can have a direct inhibitory effect on the response of Vβ6$^+$ hybridomas to the superantigen, Mls-1^a *in vitro* (17). Once injected *in vivo*, however, the TCR peptides may have an indirect effect by inducing anti-TCR-peptide T cells. The anti-peptide T cells may help a B cell response directed against the TCR, but our results on anti-peptide antibodies, in contrast to others (7, 36), indicate that if anti-Vβ6 peptide antibodies have a role in immunoregulation, their effect could not be through a direct recognition of intact TCR on T cells. Alternatively, the anti-peptide T cells may recognize the naturally processed fragment of the TCR presented by MHC molecules. The anti-peptide T cells then may directly lyse the target T cell or produce cytokines which regulate the response of the target T cell. We are currently investigating the potential regulatory function of anti-TCR-peptide T cells *in vitro* and *in vivo*.

Table 3.1 Sequences of Vβ peptides

Peptide[a)]	Sequence[b)]
Vβ6(1-20)	G G I I T Q T P K F L I G Q E G Q K L T
Vβ3(1-20)	N S K V I Q T P R Y L V K G Q G Q K A K
Vβ8.1(1-20)	E A A V I Q S P R S K V A V T G G K V T
Vβ8.3(1-20)	E A A V I Q S P R N K V T V T G G K V T
Vβ6(32-48)	M Y W Y R Q D S G K G L R L I Y Y
Vβ6(39-60)	D S G K G L R L I Y Y S I T E N D L Q K G
Vβ6(48-75)	Y S I T E N D L Q K G D L S E G Y D A S R E K K S S F S
Vβ6(58-75)	G D L S E G Y D A S R E K K S S F S

a) The peptides were synthesized according to the Merrifield solid-phase technique (17).
b) Nomenclature and sequences of peptides were taken from (1).

Table 3.2 Response to peptides of the T cell receptor in several strains of mice

Peptide	Concentration (μg/mL)	BALB/C ($H\text{-}2^d$; $Mls\text{-}1^b$)	DBA/2J ($H\text{-}2^d$; $Mls\text{-}1^a$)	CBA/CaJ ($H\text{-}2^k$; $Mls\text{-}1^b$)	CBA/J ($H\text{-}2^k$; $Mls\text{-}1^a$)	C57Bl/6J ($H\text{-}2^b$; $Mls\text{-}1^b$)	D1.LP ($H\text{-}2^b$; $Mls\text{-}1^a$)
Vβ6(1-20)[a]	0.0	1.8 ± 0.1[b]	1.2 ± 0.4	2.1 ± 0.2	2.4 ± 0.5	2.7 ± 0.4	2.6 ± 0.1
	2.5	2.7 ± 0.2	NT	3.4 ± 0.3	NT	NT	NT
	25.0	4.5 ± 0.5	2.5 ± 0.3	3.2 ± 0.6	5.8 ± 0.5	4.0 ± 0.6	2.8 ± 0.4
	250.0	5.2 ± 0.3	3.8 ± 0.5	2.7 ± 0.4	2.4 ± 0.5	NT	NT
Vβ6(32-48)	0.0	3.2 ± 0.7	9.1 ± 0.8	4.4 ± 2.2	6.0 ± 0.3	4.9 ± 0.9	
	2.5	6.5 ± 0.0	9.2 ± 1.0	4.1 ± 1.8	4.1 ± 1.1	6.2 ± 0.4	
	25.0	5.6 ± 1.7	10.3 ± 0.1	3.3 ± 1.0	8.8 ± 1.0	4.4 ± 1.8	
	250.0	24.3 ± 6.4	48.5 ± 0.4	4.1 ± 0.8	5.5 ± 1.1	4.0 ± 0.7	
Vβ6(39-60)	0.0	1.0 ± 0.1	5.4 ± 2.4	6.2 ± 0.5	5.8 ± 0.7	0.5 ± 0.2	2.5 ± 0.7[c]
	2.5	1.4 ± 0.3	4.7 ±	5.8 ± 0.7	4.2 ± 0.7	0.4 ± 0.1	2.2 ± 0.2
	25.0	1.1 ± 0.2	7.5 ± 0.9	8.2 ± 0.8	5.5 ± 0.0	0.5 ± 0.1	3.0 ± 0.9
	250.0	2.3 ± 0.5	5.4 ± 1.5	11.2 ± 0.1	12.6 ± 1.0	0.4 ± 0.1	4.9 ± 0.7
Vβ6(48-75)	0.0	1.8 ± 0.3	5.8 ± 3.1	1.5 ± 0.3	3.7 ± 0.8	1.8 ± 0.9	
	2.5	4.6 ± 0.6	12.8 ± 1.2	3.0 ± 0.8	9.6 ± 0.5	2.5 ± 0.8	
	25.0	14.0 ± 2.3	44.4 ± 12.8	7.7 ± 0.5	48.5 ± 18.0	4.1 ± 1.0	
	250.0	41.2 ± 1.0	41.9 ± 8.1	37.7 ± 3.4	101.1 ± 7.9	3.7 ± 1.3	
Vβ6(58-75)	0.0	2.8 ± 0.8	1.2 ± 0.4	3.6 ± 0.3	4.8 ± 0.5	1.8 ± 0.3	2.9 ± 0.7[c]
	2.5	7.2 ± 0.7	NT	20.3 ± 0.9	13.0 ± 2.3	NT	2.3 ± 0.3
	25.0	18.6 ± 1.2	44.8 ± 3.3	18.9 ± 2.7	14.1 ± 4.0	4.7 ± 0.4	2.5 ± 0.1
	250.0	33.2 ± 2.7	16.4 ± 18.0	18.5 ± 3.5	35.9 ± 9.1	NT	2.8 ± 0.7
Vβ3(1-20)	0.0	3.8 ± 0.0	1.5 ± 0.3	4.7 ± 0.4	7.3 ± 1.2		
	2.5	32.0 ± 1.3	NT	61.9 ± 4.0	94.1 ± 13.8		
	25.0	40.6 ± 0.8	NT	55.1 ± 7.4	91.4 ± 9.0		
	250.0	40.3 ± 7.0	2.6 ± 0.6	57.0 ± 1.7	94.2 ± 11.0		

Table 3.2 (cont'd) Response to peptides of the T cell receptor in several strains of mice

Peptide	Concentration (μg/mL)	BALB/C ($H\text{-}2^d$; $Mls\text{-}1^b$)	DBA/2J ($H\text{-}2^d$; $Mls\text{-}1^a$)	CBA/CaJ ($H\text{-}2^k$; $Mls\text{-}1^b$)	CBA/J ($H\text{-}2^k$; $Mls\text{-}1^a$)	C57Bl/6J ($H\text{-}2^b$; $Mls\text{-}1^b$)	D1.LP ($H\text{-}2^b$; $Mls\text{-}1^a$)
Vβ8.1(1-20)	0.0	0.5 ± 0.1	3.3 ± 1.0				
	2.5	0.3 ± 0.0	NT				
	25.0	0.4 ± 0.1	NT				
	250.0	0.5 ± 0.3	2.7 ± 1.0				
Vβ8.3(1-20)	0.0	0.6 ± 0.2	2.3 ± 0.0				
	2.5	0.9 ± 0.2	NT				
	25.0	1.8 ± 0.2	0.4 ± 0.2				
	250.0	2.2 ± 0.6	18.9 ± 0.6				

a) Mice were immunized with 50 μg of peptide emulsified in CFA.
b) Nylon wool purified T cells (4×10^5 cells) from primed mice were cultured with irradiated (3000 rad) syngeneic spleen cells (1×10^6 cells) with varying doses of peptide and proliferation after four days of culture was determined as described in Materials and Methods. Values presented are the avergae of triplicate cultures ± SD (cpm x 10^{-3}).
c) As above, except that 2×10^5 T cells were cultured.

Table 3.3 Frequency of Vβ6$^+$ and Vβ8$^+$ lymph node cells after *in vitro* stimulation with antigen

Peptide used in immunization[a)]	Peptide used in culture[b)]	% positive cells Vβ6	Vβ8	CD4
CFA alone	PPD[c)]	10.9	13.8	59.5
Vβ6(1-20)	PPD	9.1	13.8	58.9
Vβ6(48-75)	PPD	9.3	15.5	49.0
Vβ6(1-20)	Vβ6(1-20)	10.0	18.4	63.8
Vβ6(48-75)	Vβ6(48-75)	9.7	16.7	48.8

a) BALB/c were immunized with 50μg of peptide emulsified in CFA.
b) Microcultures were prepared as in Table 1. After 4 days of culture, viable cells were harvested and prepared for flow cytometry as described in Materials and Methods.
c) PPD is the immunogenic moiety in CFA and so was used as an antigen-specific stimulation for the negative control cultures.

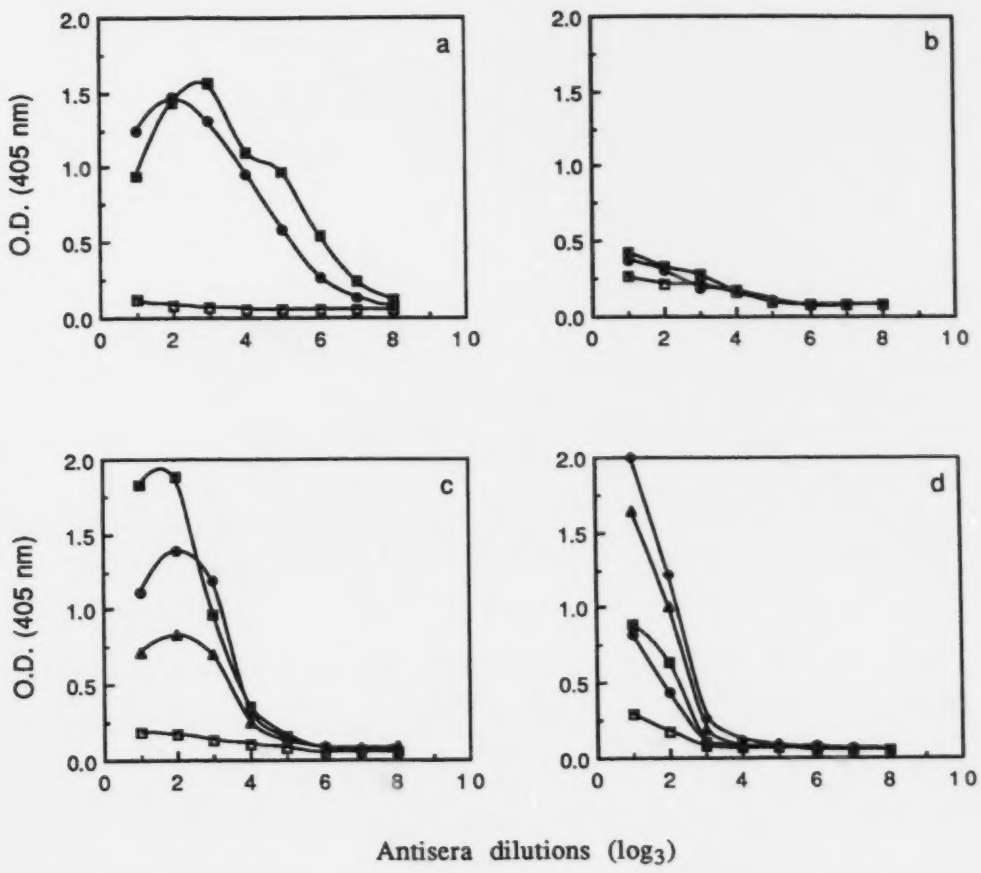

Figure 3.1. Antibody response to Vβ6 peptides by BALB/C mice. Individual Ab titres to (a) Vβ6(1-20), (b) Vβ6(32-48), (c) Vβ6(39-60), and (d) Vβ6(48-75). In all panels, open symbols are NMS and closed symbols are primed anti-peptide anti-sera.

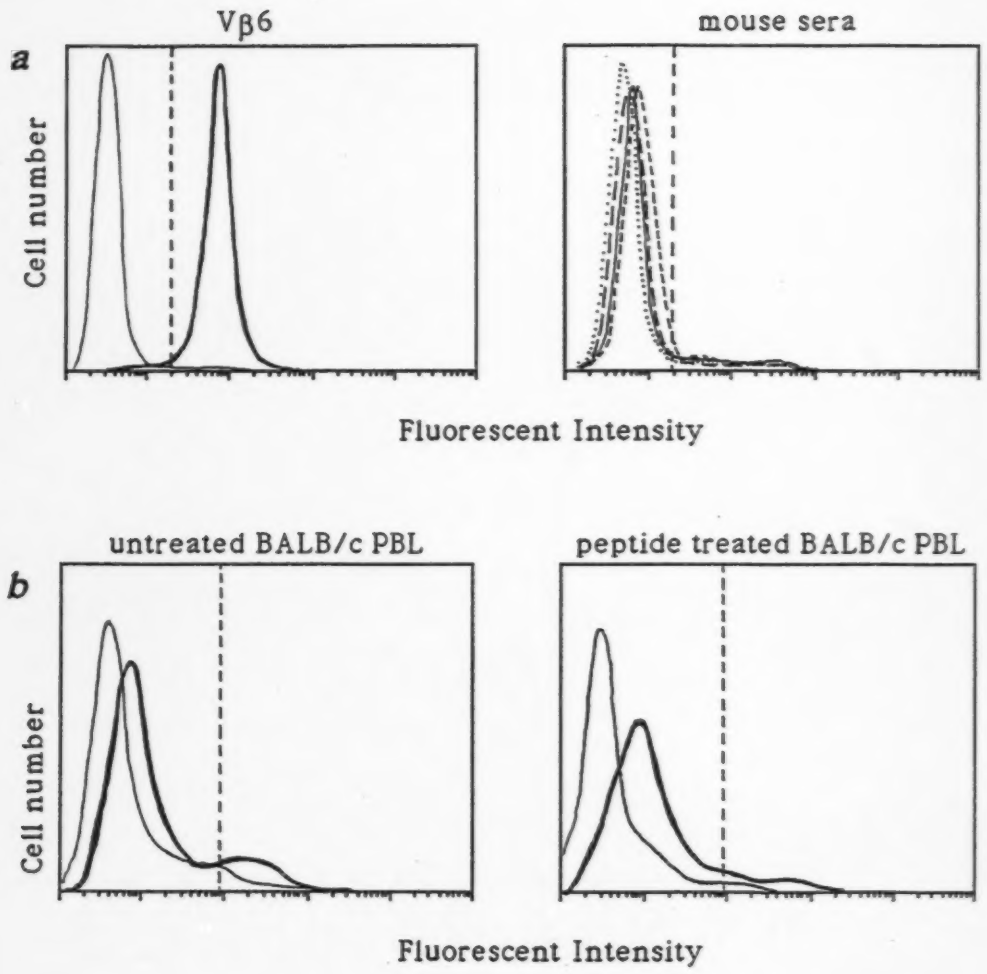

Figure 3.2(a). Anti-Vβ6 peptide sera do not bind to the intact Vβ6 TCR. A1.1 were stained with anti-Vβ6 mAb (——) or anti-Vβ8.1,2 mAb (——), followed by goat-anti-rat IgG-FITC conjugate to show TCR expression. A.1.1 were stained with NMS (·····), anti-Vβ6(1-20) serum (– – –), anti-Vβ6(48-75) serum from BALB/c mice (——) or anti-Vβ6(1-20) serum from DBA/2 mice (— —) followed by goat-anti-mouse IgG-FITC conjugate.

(b). BALB/c mice with anti-Vβ6(1-20) sera retain $V\beta6^+$ peripheral T cells. PBL were stained with anti-IL-2R mAb (——) or with anti-Vβ6 mAb (——) followed by goat-anti-rat IgG-FITC conjugate.

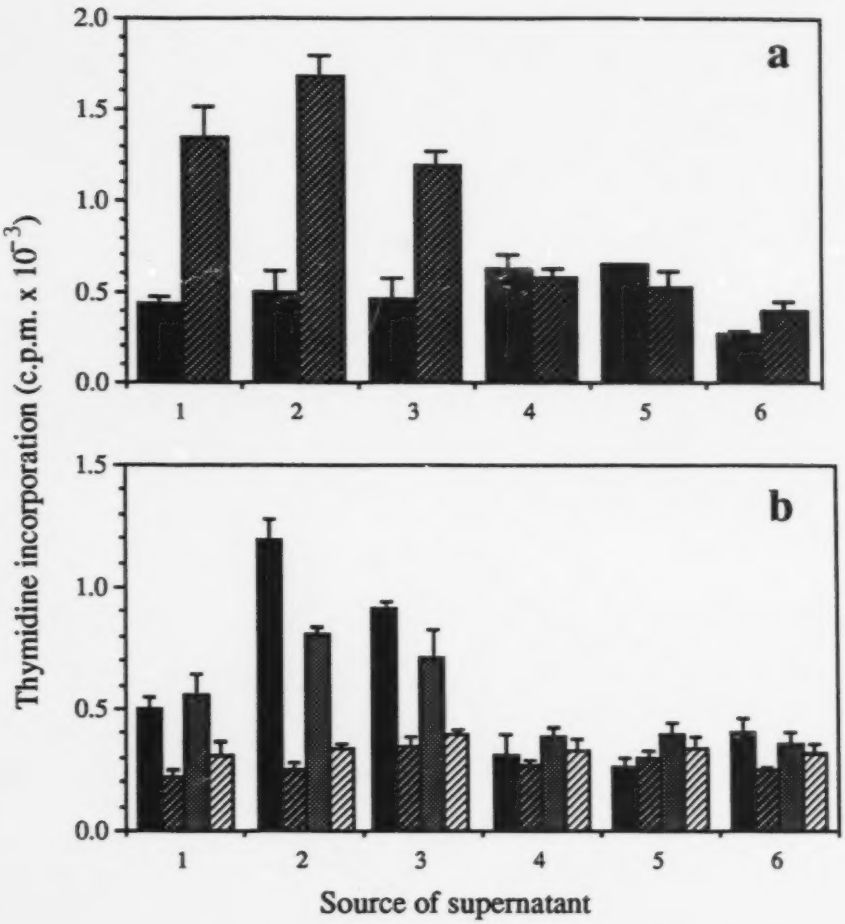

Figure 3.3 Production of IL-2 by T cells stimulated with Vβ6 peptides.
a) HT-2 cells were incubated in 50% (v/v) supernatant from T cell microcultures stimulated with APC only (■) or with APC and Vβ6(32-48) (1-3) or Vβ6(39-60) (4-6) (▨).
b) HT-2 cells were incubated in 25% (v/v) supernatant from the same cultures as above in the presence of no antibody (■), anti-IL-2 Ab (▨), anti-IL4 Ab (▩), or both antibodies (▨).

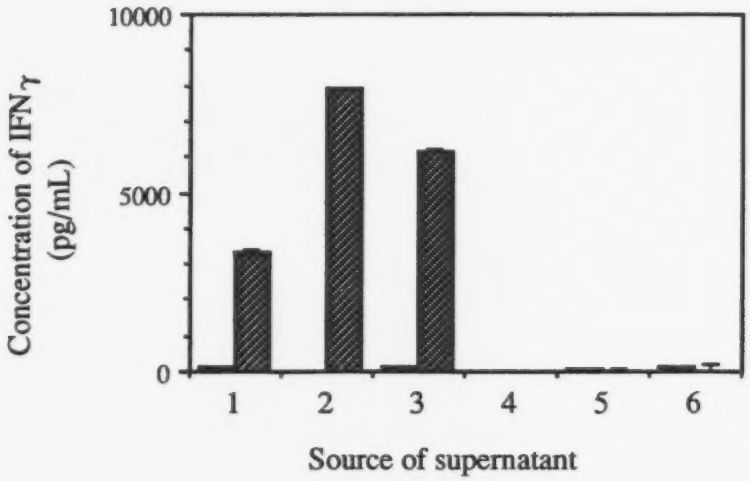

Figure 3.4 Production of IFNγ by T cells stimulated with Vβ6 peptides.
Supernatant from T cell microcultures stimulated with APC only (■) or with APC and Vβ6(32-48) (1-3) or Vβ6(39-60) (4-6) (▨) were tested for the presence of IFNγ by ELISA; absorbance was converted to pg/mL of IFNγ by comparison to a titration curve of a standard IFNγ.

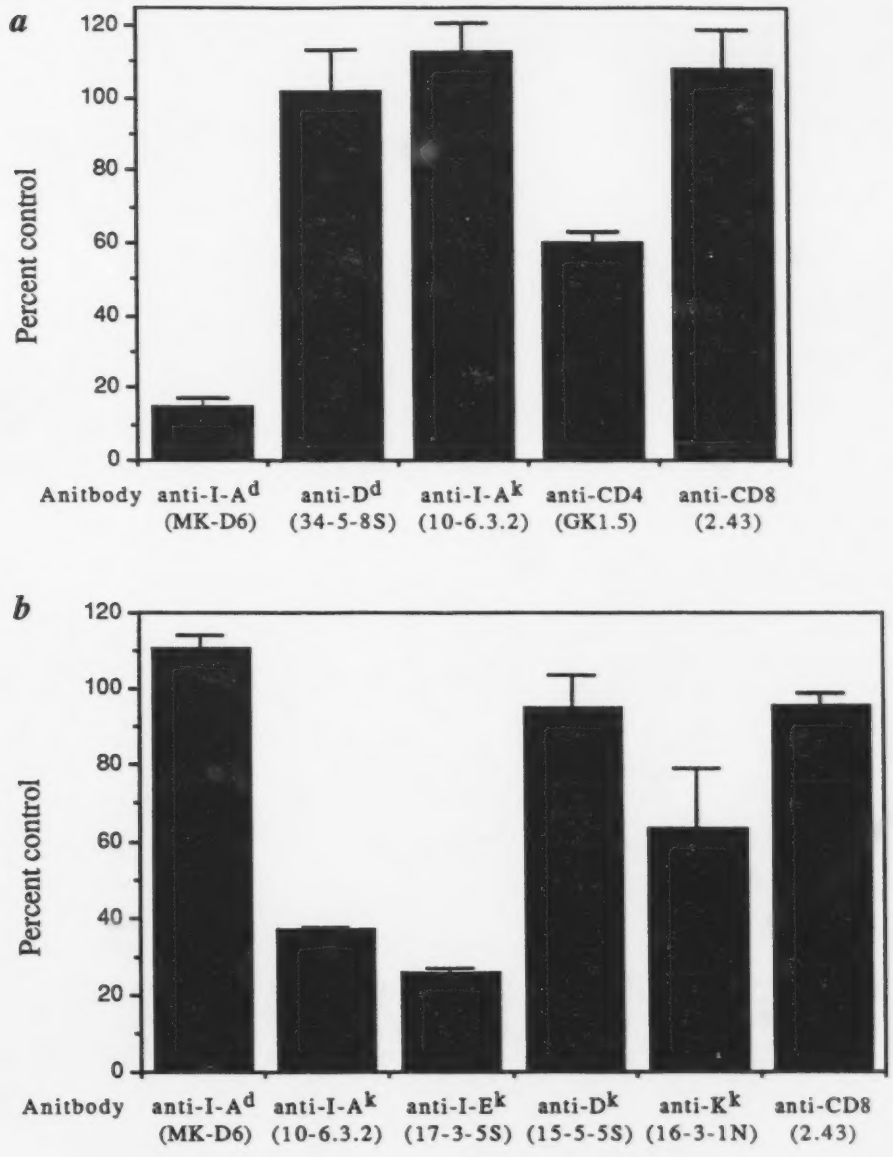

Figure 3.5. Inhibition of T cell response to Vβ6 peptides with mAb to class II MHC. Mice were immunized with 50 μg of peptide in CFA in the hind footpads. MAbs were used at a final concentration of 80 μg/mL. Values are presented as the percent of control response to the antigenic peptide in the absence of mAb. (a) Proliferation of T cells from Vβ6(1-20) primed-BALB/c in the presence of various mAb. (b) Proliferation of T cells from Vβ6(48-75) primed-CBA/CaJ in the presence of various mAb.

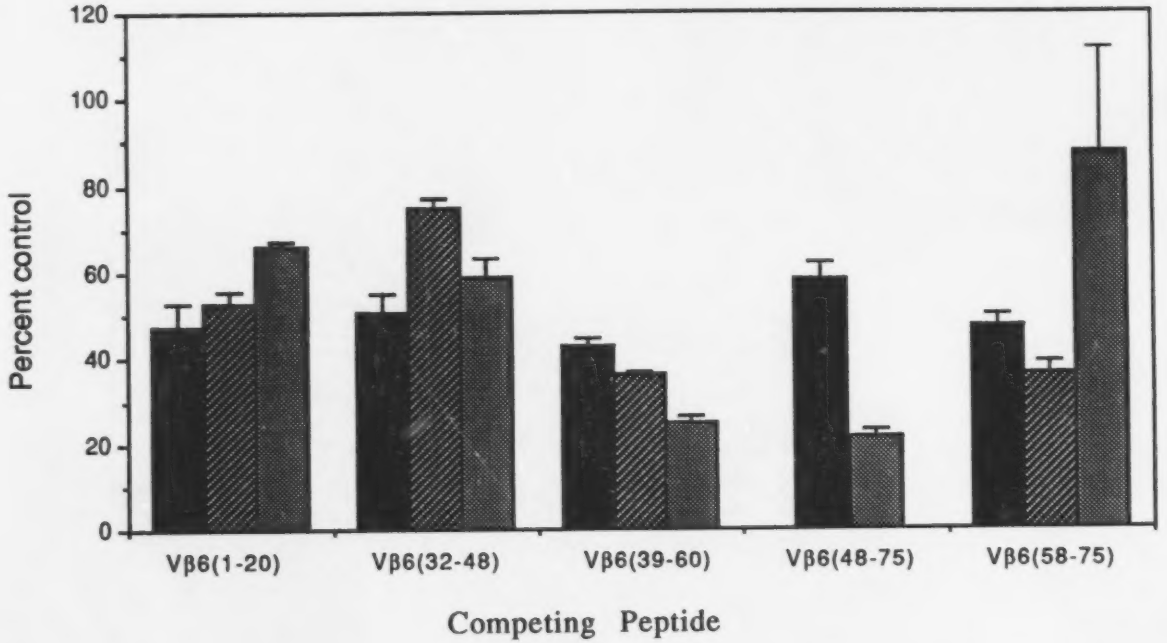

Figure 3.6. Inhibition of I-A^d restricted T cell hybridoma A.1.1 by Vβ6 peptides at various concentrations : 4 μg/mL (■), 40 μg/mL (▨), and 400 μg/mL (▩). The magnitude of the control response (in the absence of competing peptide) of A.1.1 was 14,700 ± 900 cpm in the presence of 20 μg/mL K4, and the background release of IL-2 by A.1.1 was 2,700 ± 300 cpm in the absence of K4. Fixed TA3 cells were used as APC. Values are presented as the percent of control response to the antigenic peptide in the absence of competing peptide.

Bibliography

1. Kronenberg, M., G. Siu, L.E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Ann. Rev. Immunol. 4:529.

2. Raulet, D.H. 1989. The structure, function, and molecular genetics of the γ/δ T cell receptor. Ann. Rev. Immunol. 7:175.

3. Matis, L.A. 1990. The molecular basis of T-cell specificity. Ann. Rev. Immunol. 8:65.

4. White, J., A. Herman, A.M. Pullen, R. Kubo, J.W. Kappler, and P. Marrack. 1989. The Vβ-specific superantigen *staphylococcol* enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell 56:27.

5. MacDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha-Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. Nature 332:40.

6. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541.

7. Offner, H., G.A. Hashim, and A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomuelitis. Science 251:430.

8. Desquenne-Clark, L., T.R. Esch, L. Otvos, Jr., and E. Heber-Katz. 1991. T-cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis. Proc. Natl. Acad. Sci. USA 88:7219.

9. Ramsdell, F., and B.J. Fowlkes. 1990. Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. Science 248:1342.

10. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature 332:35.

11. Festenstein, H., S. Kumura, and G. Biasi. 1989. Mls and tolerance. Immunol. Rev. 107:30.

12. Frankel, W.N., C. Rudy, J.M. Coffin, and B.T. Huber. 1991. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. Nature 349:526.

13. Abe, R., M. Foo-Phillips, and R.J. Hodes.1991. Genetic analysis of the Mls system. Formal Mls typing of the commonly used inbred strains. Immunogenetics 33:62.

14. Okada, L.Y., B. Holzmann, C. Guidos, E. Palmer, and I.L. Weissman. 1990. Characterization of a rat monoclonal antibody specific for a determinant encoded by the Vβ7 gene segment. Depletion of Vβ7$^+$ T cells in mice with Mls-1^a haplotype. J. Immunol. 144:3473.

15. Happ, M.P., D.L. Woodland, and E. Palmer. 1989. A third T-cell receptor β-chain variable region gene encodes reactivity to Mls-1^a gene products. Proc. Natl. Acad. Sci. USA 86:6293.

16. Abe, R., M.S. Vacchio, B. Fox, and R.J. Hodes. 1988. Preferential expression of the T-cell receptor Vβ3 gene by Mls^c reactive T cells. Nature 335:827.

17. MacNeil, D., E. Fraga, and B. Singh. 1992. Inhibition of superantigen recognition by Vβ peptides. Eur. J. Immunol. 22:937-941.

18. Lee, K.C., B. Singh, M.A. Barton, A. Proyshyn, and M. Wong. 1979. A simple reliable system for studying antigen-specific murine T cell proliferation. J. Immunol. Methods 25:159.

19. Singh, B., C.A. Waters, and D. Swanlund. 1986. Alloantigenic sites on class I major histocompatibility complex antigens. I. 61-69 region of the first domain of the H-2K^b molecules

induces specific antibody and T cell responses. J. Immunol. 137:2311.

20. Fotedar, A., M. Boyer, W. Smart, J. Widtman, E. Fraga, and B. Singh. 1985. Fine specificity of antigen recognition by T cell hybridoma clones specific for poly-18: a synthetic polypeptide antigen of defined sequence and conformation. J. Immunol. 135:3028.

21. Boyer, M., Z. Novac, E. Fraga, K. Oikawa, C.M. Kay, A. Fotedar, and B. Singh. 1990. Functional degeneracy of residues in a T cell peptide epitope contribute to its recognition by different T cell hybridomas. Int. Immunol. 2:1221.

22. Glimcher, L.H., T. Hamano, R. Asofsky, E. Heber-Katz, S. Hedrick, R.H. Schwartz, and W.E. Paul. 1982. I region restricted antigen presentation by B cell-B lymphoma hybridomas. Nature 298:283.

23. Vandenbark, A.A., T. Gill, and H. Offner. 1985. A myelin basic protein-specific T lymphocyte line that mediates experimental autoimmune encephalomyelitis. J. Exp. Med. 135:223.

24. Schild, H., O. Fotzschke, H. Kalbacher, and H.-G. Rammensee. 1990. Limit of T cell tolerance to self proteins by peptide presentation. Science 247:1587.

25. Benichou, G., P.A. Takizawa, P.T. Ho, C.C. Killion, C.A. Olson, M. McMillan, and E.E. Sercarz. 1990. Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides. J. Exp. Med. 172:1341.

26. Agrawal, B., M. Manickasundari, E. Fraga, and B. Singh. 1991. T cells that recognize peptide sequences of self MHC class II molecules exist in syngeneic mice. J. Immunol. 147:383.

27. Schneider, R., R.K. Lees, T. Pedrazzini, R.M. Zinkernagel, H. Hengartner, and H.R. MacDonald. 1989. Postnatal disappearance of self-reactive (Vβ6+) cells from the thymus

of Mls^a mice. Implications for T cell development and autoimmunity. J. Exp. Med. 169:2149.

28. Hengartner, H., B. Odermatt, R. Schneider, M. Schreyer, G. Walle, H.R. MacDonald, and R.M. Zinkernagel. 1988. Deletion of self-reactive T cells before entry into the thymus medulla. Nature 336:388.

29. Oki, A. and E. Sercarz. 1985. T cell tolerance studied at the level of antigenic determinants. I. Latent reactivity to lysozyme peptides that lack suppressogenic epitopes can be revealed in lysozyme-tolerant mice. J. Exp. Med. 161:897.

30. Salgame, P., J.S. Abrams, C. Clayberger, H. Goldstein, J. Convit, R.L. Modlin, and B.R. Bloom. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science 254:279-282.

31. Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348-2357.

32. Scott, P., P. Natovitz, R.L. Coffman, E. Pearce, and A. Sher. 1988. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protictive immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. J. Exp. Med. 168:1675-1684.

33. Ashbridge, K.R., B.T. Backstrom, H.-X. Li, T. Vikerfors, D.R. Englebretsen, D.R.K. Harding, and J.D. Watson. 1992. Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of *Mycobacterium tuberculosis*: evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity responses. J. Immunol. 148:2248-2255.

34. Adorini, L., M.A. Harvey, A. Miller, and E.E. Sercarz. 1979. Fine specificity of regulatory T cells. II. Suppressor and helper T cells are induced by different regions of hen egg-white lysozyme in a genetically nonresponder mouse strain. J. Exp. Med. 150:293-306.

35. Howell, M.D., S.T. Winters, T. Olee, H.C. Powell, D.J. Carlo, and S.W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. Science 246:668.

36. Hashim, G.A., A.A. Vandenbark, A.B. Galang, T. Diamanduros, E. Carvalho, J. Srinivasan, R. Jones, M. Vainiene, W.J. Morrison, and H. Offner. 1990. Antibodies specific for Vβ8 receptor peptide suppress experimental autoimmune encephalomyelitis. J. Immunol. 144:4621.

37. Goodnow, C.C., S. Adelstein, and A. Basten. 1990. The need for central and peripheral tolerance in the B cell repertoire. Science 248:1373.

38. Sette, A., S. Buus, E. Appella, J.A. Smith, R. Chesnut, C. Miles, S.M. Colon, and H.M. Grey. 1989. Prediction of major histocompatibility complex binding regions of protein antigens by sequence pattern analysis. Proc. Natl. Acad. Sci. USA 86:3296.

39. Lorenz, R.G. and P.M. Allen. 1988. Direct evidence for functional self protein/Ia molecule complexes in vivo. Proc. Natl. Acad. Sci. USA 85:5220.

40. Rosloniec, E.F., L.J. Vitex, S. Buus, and J.H. Freed. 1990. MHC class II-derived peptides can bind to class II molecules, incuding self molecules, and prevent antigen presentaion. J. Exp. Med. 171:1419.

41. Roehm, N.W., A. Carbone, E. Kushnir, B.A. Taylor, R.J. Riblet, P. Marrack, and J.W. Kappler. 1985. The major histocompatibility complex-restricted antigen receptor on T cells: the genetics of expression of an allotype. J Immunol. 135:2176.

42. Prehn, R.T. 1990. Immunological tolerance to many self epitopes may be unnecessary. Scand. J. Immunol. 32:293.

43. von Boehmer, H. and P. Kisielow. 1990. Self - nonself discrimination by T cells. Science 248:1369.

44. Nikolic-Zugic, J. and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. Nature 344:65.

45. Fry, A.M., M.M. Cotterman, and L.A. Matis. 1989. The influence of self-MHC and non-MHC antigens on the selection of an antigen-specific T cell receptor repertoire. J. Immunol. 143:2723.

46. Kawano, Y.-I., Y. Sasamoto, S. Kotake, S.R. Thurau, B. Wiggert, and I. Gery. 1991. Trials of vaccination against experimental autoimmune uveoretinitis with a T-cell receptor peptide. Curr. Eye Res. 10:789.

CHAPTER IV

Effect of pre-immunization with self peptides of the TCR on the T cell response to superantigen, peptide antigen, and on the transfer of diabetes in NOD mice

Introduction

Regulation of immune responses is needed to minimize damage to host tissues during infection and to maintain tolerance to self. Regulation is achieved by a variety of means: selection of T cells and B cells, cytokines, and perhaps a cellular network of cooperation and suppression (1). Evidence for a network of T cells can be found in the modulation of immune responses by immunization with activated T cells specific for self antigen (2) or alloantigen (3, 4). The immunized animal is subsequently rendered tolerant to the antigen, perhaps through the induction of anti-idiotypic T cells (5). These findings are consistent with the proposal that peptides derived from the T cell receptor (TCR) and immunoglobulins may play a regulatory role within the immune system (6).

Further support of this is the protection from experimental autoimmune encephalomyelitis (EAE) in rats, by immunization with a peptide from the variable region of the β chain of a TCR,

Vβ8.2(39-59) (7, 8). EAE is mediated by Vβ8.2$^+$ T cells specific for myelin basic protein (MBP) (9). T cells specific for the Vβ8.2-peptide somehow inhibit the function or induction of Vβ8.2$^+$ encephalitogenic T cells (7, 8). However, this protective effect of the Vβ8.2-peptide has not been reproduced in other studies (10, 11). In addition to the effectiveness being questionable, the effect of immunization with TCR-peptides has not been applied to other T cell responses.

We have chosen to examine this phenomenon in other systems which involve recognition of superantigen, peptide antigen, and self antigen by different TCR in mice. Specifically, the responses to endogenous superantigen Mls-1^a, and a synthetic peptide $(EYA)_5$ by BALB/c mice, are both dominated by T cells expressing Vβ6 (12, 13). We have observed that several peptides of the TCR-Vβ6 are immunogenic in many strains of mice (14), and so have the potential to modulate immune responses. Here we examine the effect of pre-immunization with Vβ6-peptides on the T cell responses to Mls-1^a and to $(EYA)_5$. Since the T cell responses to these antigens involve predominant usage of Vβ6, immunization with peptides of Vβ6 may lead to down-regulation of the response to Mls-1^a or $(EYA)_5$.

In addition, we examine another autoimmune disease mediated by T cells, Type I diabetes in NOD mice. It has been shown that pre-immunization with CFA can protect NOD mice from spontaneous

development of diabetes (15), and from the transfer of diabetes by the co-injection of splenocytes from CFA-protected mice with splenocytes from diabetic mice (16). We tested the effectiveness of splenocytes from NOD mice immunized with Vβ3-peptides in protecting recipients from the transfer of diabetes. Vβ3-peptides were chosen since $V\beta3^+$ T cells are observed among the heterogeneous population of lymphocytes which infiltrate pancreatic islets (17 - 19), in spite of the thymic deletion of $V\beta3^+$ T cells in NOD mice by an endogenous superantigen, mtv-3 (20). Although the Vβ3-peptides do not protect recipients from the transfer of diabetes, we observe a small, but statistically significant, reduction in the T cell *in vitro* proliferative response to $Mls\text{-}1^a$ and $(EYA)_5$ upon pre-immunization with some Vβ6-peptides. Thus peptides of the TCR may modulate many types of T cell responses.

Materials and Methods

Mice.

DBA/2J, CBA/CaJ, and CBA/J mice were purchased from the Jackson Laboratory, Bar Harbor, ME. BALB/cCr and NOD mice were bred at the Ellerslie Animal Farm of the University of Alberta. Diabetic NOD mice were maintained with daily injections of 50 μL of Ultralente Insulin (NOVO Labs LTD., Willowdale, Ontario).

Peptides.

Peptides were prepared by the Merrifield solid-phase technique on a Beckman 990C Peptide Synthesizer (Palo Alto, CA), as previously described (21, Chapter II).

Immunizations.

Mice were immunized in the footpads with 50 μg of peptide emulsified with CFA (Sigma, St. Louis, MO). Mice used in the MLR experiments against Mls-1^a superantigen were taken eight or forty days later, and draining popliteal lymph node cells were prepared for the T cell proliferation assay. BALB/c mice used in the response to $(EYA)_5$ were immunized in the hind leg with 50 μg of $(EYA)_5$ emulsified with IFA (Sigma), eight days after the first immunization with Vβ6 peptide. After an additional eight days, cells from popliteal lymph nodes were prepared as described below. NOD mice were immunized in the foot pad with 50 μg of peptide emulsified with IFA. Eight days later, spleen cells and popliteal lymph nodes were pooled before injection into recipient NOD mice.

MLR.

Cells of popliteal lymph nodes from immunized BALB/c or CBA/CaJ mice were purified over nylon wool columns (22) and then cultured in 96-well plates (Flow, McLean, VA) at 2 x 10^5, 1 x 10^5, 0.5 x 10^5, 0.25 x 10^5, and 0.125 x 10^5 cells per well with 10 x 10^5, 5.0 x 10^5, 2.5 x 10^5, 1.25 x 10^5, or 0.62 x 10^5 DBA/2J or CBA/J stimulator spleen cells in complete medium (RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FCS (Bocknek, Canada), 10 mM Hepes, 2 mM L-glutamine, 5x10^{-5} M 2-ME, and 1 U/ml penicillin-streptomycin). The stimulator spleen cells were first treated with 20 µg/mL of Mitomycin C (Sigma) for 30 minutes at 37°C, and washed thoroughly with medium before culture. The MLR cultures were incubated for four days at 37°C, and 7% CO_2, then pulsed with 37 kBq of [^{3}H]dThd (NEN Dupont, Boston, MA) for 16-20 h. Incorporation of [^{3}H]dThd was measured using either a liquid scintillation counter (LKB Instruments, Gaithersburg, MD) or a gas scintillation Matrix96 (Canberra Packard Canada, Mississauga, Ontario). At each concentration of responding T cells, the c.p.m. values for each group were standardized to the CFA only group response to 1 x 10^6 stimulating cells, and expressed a percentage of the control response according to the following formula:

$$\frac{\text{c.p.m. of peptide treated group}}{\text{c.p.m. of CFA control group at 1 x } 10^6 \text{ APC}} \times 100\%$$

T cell proliferation assay for response to peptide antigen.

Purified cells from immunized mice were cultured in 96-well plates in complete media (described above) at 4 x 10^5 or 2 x 10^5 cells/well with syngeneic irradiated (3000 rad) spleen cells as APC at 1 x 10^6 cells/well in the presence or absence of peptide in 200 µL of culture medium. Concentrations of peptides tested were 250 µg/mL, 25 µg/mL, and 2.5 µg/mL; PPD (25 µg/mL) served as a positive control for T cell proliferation. Incorporation of [^{3}H]dThd was measured as described above. In the response to $(EYA)_5$, the c.p.m. values for each peptide treated group were standardized to the CFA only group response to 25 µg/mL $(EYA)_5$, and expressed a percentage of the control response according to the following formula:

$$\frac{\text{c.p.m. of peptide treated group}}{\text{c.p.m. of CFA control group at 25 µg/mL } (EYA)_5} \times 100\%$$

Statistical analysis.

Where appropriate, data from different experiments were pooled for each treatment group. Means and standard deviations for each group were calculated, and the significant differences of the treated groups from that of the CFA control group was measured by an analysis of variance (23). Differences were considered significant at $p \leq 0.05$.

Transfer of diabetes in NOD mice.

The transfer of diabetes by injection of splenocytes from diabetic NOD mice into irradiated recipients has been previously described (16). Briefly, recipient female NOD mice (7 - 9 weeks old) were irradiated (850 rad) and injected with a total of 30 x 10^6 cells intravenously. Cells were prepared as follows. Spleen cells from diabetic NOD mice were depleted of RBC by a 3 minute incubation with Tris-buffered ammonium chloride (0.16 M NH4Cl, 0.17 M Tris) and washed thoroughly. Diabetic spleen cells were mixed in a 1:1 ratio with pooled spleen and lymph node cells from immunized NOD mice. Twelve days after injection, recipients were monitored for urine glucose levels with Tes-Tape (Eli Lilly Canada Inc, Toronto, Ontario) until urine glucose levels were above normal, then blood glucose was monitored every second day with a Glucoscan 2000 (Lifescan Inc., Mountain View, CA). The recipients were considered to be diabetic when blood glucose levels were greater than 300 mg/dL. Recipient NOD mice were sacrificed by CO_2 when diabetes was confirmed by two consecutive blood glucose readings greater than 300 mg/dL.

Results

Response to superantigen.

The response to DBA/2J by BALB/c T cells is dominated by Vβ6$^+$ T cells (13). Thus if immunization with peptides of the Vβ6 region of the TCR can inhibit the response of Vβ6$^+$ T cells, then a difference in the proliferation of anti-Mls-1^a T cells should be detectable. In order to assess the effect of peptides of the Vβ-TCR on T cells responses, the response of T cells from BALB/c or CBA/CaJ mice to DBA/2J and CBA/J stimulatory cells respectively, was measured following immunization of the responder mice with Vβ6-peptides or saline emulsified in CFA. Table 4.1 lists the peptides used in this study; these peptides have been previously shown to induce T cell proliferative responses in BALB/c and CBA/CaJ (14). Eight or forty days after immunization, the draining popliteal lymph nodes of BALB/c or CBA/CaJ mice were purified over nylon wool columns and co-cultured with varying densities of DBA/2J or CBA/J spleen cells, which had been treated with Mitomycin C. Because no difference in patterns of response could be detected between 8 and 40 days after immunization, Figure 4.1 presents the pooled data from three experiments of different groups of BALB/c at different densities of responding T cells: 0.25×10^5 (Fig. 4.1a-c), 0.5×10^5 (Fig. 4.1d-f), 1.0×10^5 (Fig. 4.1g-i), and 2.0×10^5 (Fig. 4.1j-l). Responses within groups can be quite variable and all groups of BALB/c have similar proliferative responses to DBA/2J APC at most concentrations. At the lower densities of responding T cells the groups immunized with Vβ6(1-20) or Vβ6(58-75) show slightly

reduced proliferations (Fig. 4.1a, d-f). At the highest density of T cells, reduced proliferation is evident only in the group immunized with Vβ6(48-75) (Fig. 4.1j-l).

Only those Vβ6-peptides which are immunogenic in CBA/CaJ as measured by T cell proliferation *in vitro*, Vβ6(48-75) and Vβ6(58-75), were tested for an effect on the response of CBA/CaJ mice to Mls-1^a. Similarly for CBA/CaJ anti- CBA/J MLR, differences in the proliferation of responding T cells are evident at only some densities of cells, with greatest differences occurring at the higher densities of responding T cells (Fig. 4.2g-l). Unlike the BALB/c anti-DBA/2J MLR, differences were seen between 8 days versus 40 days following immunization with Vβ6-peptides. In the latter group, much less reduction in proliferation was seen, especially in the Vβ6(58-75) group. Either the effect of Vβ6-peptides appears to be lost between 8 and 40 days post-immunization, or the effect is variable and was not evident in the second set of mice.

Response to peptide antigen.

The effect pre-immunization with selected Vβ6-peptides on the T cell response to peptide antigen was also examined. Vβ6(32-48) was chosen as an additional negative control since it showed no inhibitory effect in previous experiments, and Vβ6(48-75) was chosen since it most consistently inhibited T cell responses to the superantigen, Mls-1^a. The responding T cell repertoire to Poly-18 and $(EYA)_5$ by BALB/c mice is composed of 50% Vβ6$^+$ T cells (12).

Therefore, as with the response to Mls-1^a, downregulation of this subset of T cells is expected to lead to a reduction in proliferation in response to $(EYA)_5$. Fig. 4.3 shows the pooled results from two experiments. In all but the lowest concentration of $(EYA)_5$, the proliferation of the Vβ6(48-75) pre-immunized group is slightly lower than that of the CFA control group, however this difference is significant ($p<0.01$) only at the highest concentration of $(EYA)_5$, 250 μg/mL.

Transfer of diabetes in NOD mice.

Since diabetes can be transferred to young, naive NOD mice by injection of splenocytes from diabetic NOD mice (24), the effect of splenocytes primed by Vβ3-peptides on the transfer of diabetes *in vivo* was examined. Peptides of Vβ3 were chosen since, in spite of the deletion of Vβ3$^+$ T cells in NOD mice (20), Vβ3$^+$ T cells are observed among lymphocytes infiltrating pancreatic islets at a higher frequency than found in lymph nodes of pre-diabetic NOD mice (18, 19). However, before testing this, the immunogenicity of the Vβ3-peptides was determined. T cells purified from popliteal lymph nodes of primed NOD mice proliferate in response to Vβ3(38-59), but not to Vβ3(1-20) *in vitro*, regardless of the adjuvant used in priming (Table 4.2). Since NOD mice do not respond to Vβ3(1-20), this peptide was expected to have no effect on the transfer of diabetes. The incidence of the transfer of diabetes was unaffected by splenocytes primed by either Vβ3 peptide (Fig. 4.4). Also the average time for the onset of diabetes

after transfer of splenocytes was unchanged: 20 days for both peptide groups compared to 19 days for the IFA control group. Thus there is no evidence that Vβ3-peptide primed splenocytes can inhibit the transfer of diabetes in NOD mice.

Discussion

One prediction of the network theory is that lymphocytes which recognize fragments of TCRs or Abs can regulate the response of other lymphocytes bearing those receptors. We have generated T cells specific for peptides of the TCR by immunization with peptides of the Vβ6 region of the TCR in order to test their effect on T cell responses which predominantly involve Vβ6$^+$ T cells. The T cell response to Mls-1^a *in vitro* by mice pre-immunized with Vβ6- peptides is slightly reduced in only some densities of responding and stimulating cells. This is difficult to explain. A mixture of T cells including Vβ6$^+$, Vβ8.1$^+$, Vβ7$^+$, and Vβ9$^+$ T cells (25 - 28), are able to respond to Mls-1^a and this heterogeneous population may not respond in a simple linear fashion that one might expect from a clonal population. The reduction in proliferation appears more pronounced in the CBA/CaJ response to Mls-1^a than in the BALB/c response. This may be because BALB/c and DBA/2J mice differ at more non-MHC loci than do CBA/CaJ and CBA/J mice. Thus some of the proliferative response could be due to alloantigens distinct from Mls and this portion of the response would not be affected by Vβ6- peptides.

Again for the response to peptide antigen, $(EYA)_5$, there was observed a significant inhibition of proliferation only at one concentration of antigen. Perhaps at the lower concentrations of $(EYA)_5$, antigen is limiting, and therefore not all the potentially responsive T cells are proliferating, even in the control group with

CFA only. At the highest concentration, where antigen is likely not limiting, there are fewer T cells with the potential to respond in the peptide-treated group resulting in a lower uptake of [^{3}H]dThd compared to the control group with CFA only. Others have reported similar results in that pre-immunization with peptides derived from Vβ8.2 can reduce responsiveness to myoglobin, characterized by a predominance of Vβ8.2 usage (29).

The inhibitory effect on the responses to superantigen and peptide antigen are small, probably because of the heterogeneous group of T cells which respond to the antigens. The Vβ6-peptides are expected to affect only the portion of the responding T cells which are Vβ6$^+$. In terms of the total response to antigen, immunizing with a peptide of one TCR-Vβ would significantly reduce a T cell response only if that response is homogeneous for that TCR-Vβ. However, the small reduction in response to Mls-1^a and $(EYA)_5$ demonstrated in this study shows that Vβ-peptides injected *in vivo* can modulate more heterogeneous responses and supports the theory that Vβ–peptides may play a regulatory role in immune responses of Vβ6$^+$ T cells.

Neither Vβ3 peptide tested, even Vβ3(38-59) which is immunogenic in NOD mice, had any effect on the *in vivo* induction of diabetes. Vβ8.2(39-59) has been shown to inhibit the induction of EAE in rats (7), which is mediated by Vβ8$^+$ T cells. Conversely, others have shown that Vβ8.2(39-59) has no protective effect (10),

and can actually enhance EAE (11). Although Vβ3$^+$ T cells are observed among infiltrating lymphocytes in islets of NOD mice in spite of the thymic deletion of these T cells in NOD mice (20), other T cells may be responsible for islet destruction since T cells bearing many different Vβs can be found among infiltrating lymphocytes (17, 18). Thus the ineffectiveness of Vβ3-peptides in these experiments may be due to the heterogeneity of T cells involved in the induction of diabetes.

The mechanism by which response to superantigen and peptide antigen may be inhibited by Vβ peptides is uncertain. Probably the TCR peptides have an indirect effect by inducing anti-TCR-peptide T cells. The network theory, originally proposed for interactions among idiotypic and anti-idiotypic antibodies (1), may extend to T cells which recognize fragments of TCR and Ig (6). Although the T cell response to the TCR-peptides is primarily by CD4$^+$, class II-restricted T cells (14), the TCR-peptides may also induce a small proportion of class I-restricted T cells capable of regulating other T cells. Attempts to expand *in vitro* any class I-restricted T cells specific for the Vβ6-peptides have not been successful, however, anti-K^k MAb (14), and anti-CD8 MAb inconsistently (unpublished observation), reduce the proliferation of CBA/CaJ T cells in response to CBA/J APC. T cells are capable of presenting antigen (30), and may present peptide fragments of their own TCR. TCR-peptide-specific T cells could recognize processed fragments of the TCR molecules on the cell surface of other T cells, and regulate the

function of the target T cells. Thus the reduction in proliferation of the responding T cells from mice immunized with Vβ6-peptides, may be due to suppression or anergy of the $V\beta6^+$ T cells. In conclusion, pre-immunization with TCR peptides can partially inhibit the T cell response to superantigen and peptide antigen, indicating that T cells specific for TCR peptides may have a role in the regulation of T cell responses.

Table 4.1 Sequences of peptides used in this study

Peptide[a)]	Sequence[b)]
Vβ6(1-20)	GGIITQTPKFLIGQEGQKLT
Vβ6(32-48)	MYWYRQDSGKGLRLIYY
Vβ6(48-75)	YSITENDLQKGDLSEGYDASREKKSSFS
Vβ6(58-75)	GDLSEGYDASREKKSSFS
Vβ3(1-20)	NSKVIQTPRYLVKGQGQKAK
Vβ3(38-59)	NKNNEFKFLINFQNQEVLQQID
$(EYA)_5$	EYAEYAEYAEYAEYA

a) The peptides were synthesized according to the Merrifield solid-phase technique (21).
b) Nomenclature and sequences of peptides were taken from (31).

Table 4.2. Response by primed NOD mice to peptides of Vβ3 T cell receptor

Peptide	Concentration (μg/mL)	Emulsified in[a)] CFA	IFA
Vβ3(1-20)	0.0	0.1± 0.0	0.2± 0.1
	2.5	0.1± 0.0	0.2± 0.0
	25.0	0.1± 0.0	0.2± 0.1
	250.0	0.2± 0.1	0.2± 0.1
PPD[b)]	25.0	17.3± 0.8	4.0± 0.5
Vβ3(38-59)	0.0	0.3± 0.1	0.3± 0.1
	2.5	1.2± 0.7	2.9± 0.5
	25.0	1.8± 0.8	2.7± 0.1
	250.0	4.4± 1.0	4.8± 0.7
PPD	25.0	22.6± 1.1	5.4± 1.1

a) NOD mice were immunized with peptides emulsified in CFA or IFA. After 8 days, purified cells of popliteal lymph nodes were cultured at 4 x 10^5 cells/well with 1 x 10^6 APC and different concentrations of peptides. Incorporation of [^{3}H]dThd was measured after 4 days.
b) PPD served as a positive control for T cell proliferation.

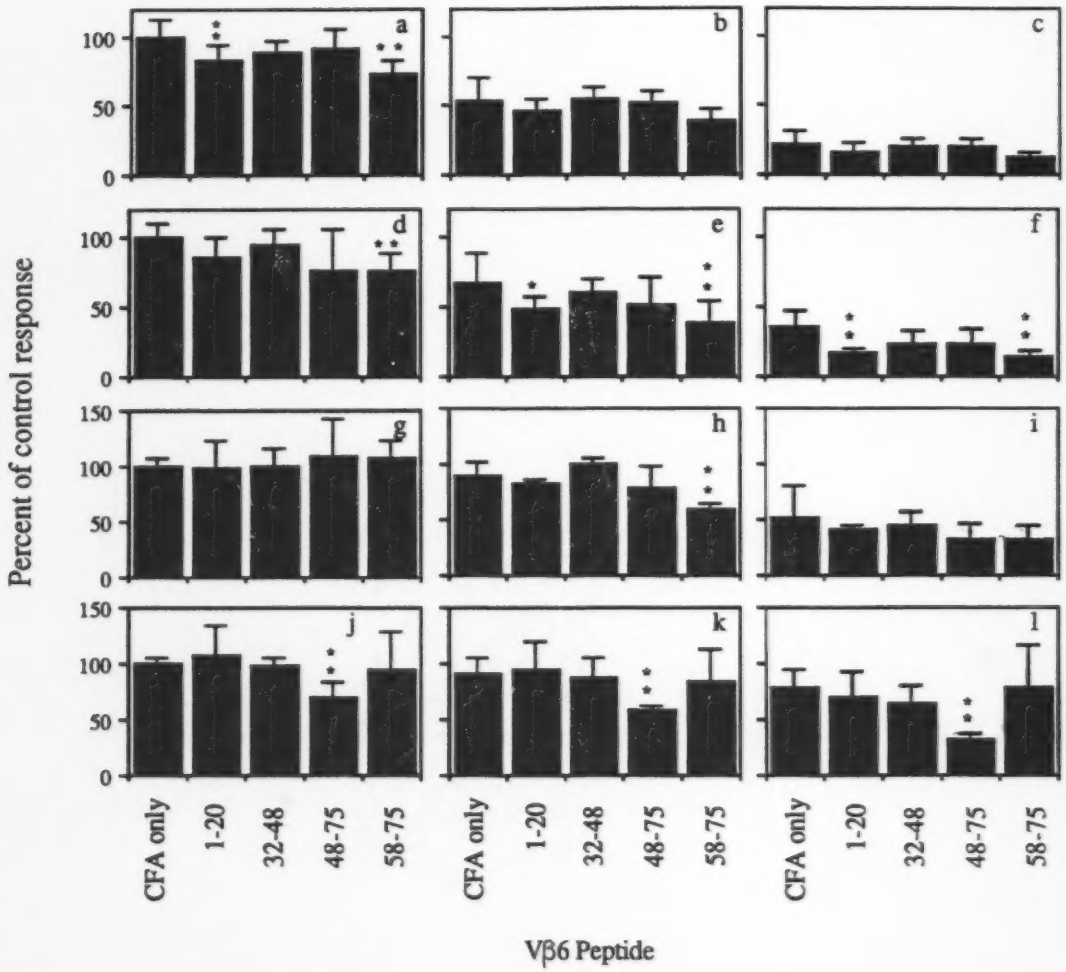

Figure 4.1. Effect of immunization with Vβ6 peptides on BALB/c mice response to Mls-1^a (DBA/2J splenocytes).
T cells were purified from popliteal lymph nodes of BALB/c mice immunized with saline or peptide emulsified in CFA. T cells were cultured at 0.25 x 10^5 (a - c), 0.5 x 10^5 (d - f), 1.0 x 10^5 (g - i) or 2.0 x 10^5 (j - l) cells with 1 x 10^6 (a, d, g, j), 0.5 x 10^6 (b, e, h, k), or 0.25 x 10^6 (c, f, i, l) DBA/2J splenocytes in 200 µL culture medium. Incorporation of [^{3}H]dThd was measured as described in Materials and Methods, and each cpm value was converted to the percent of the saline/CFA control response to 1 x 10^6 APC. (* indicates $p< 0.05$, and ** indicates $p< 0.01$ in an analysis of variance)

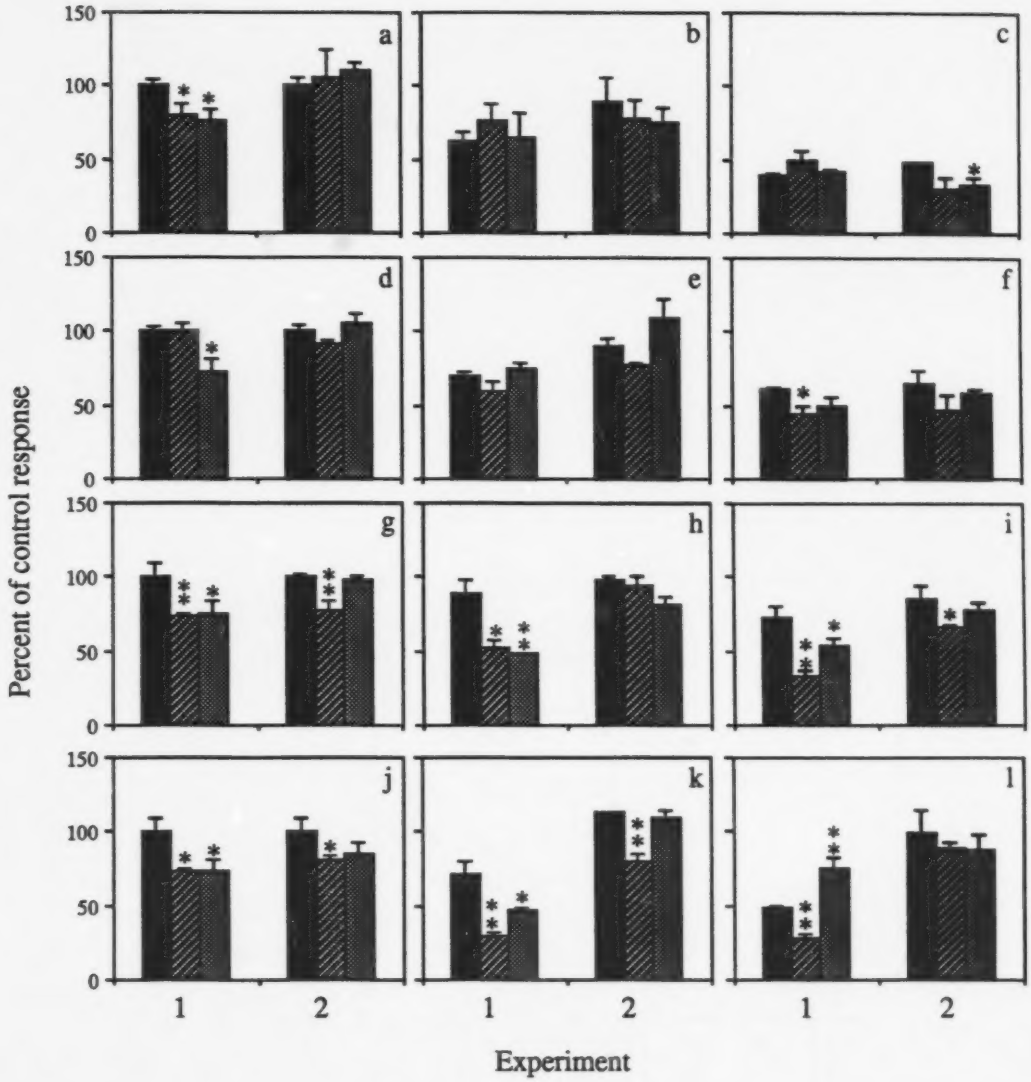

Figure 4.2. Effect of immunization with Vβ6 peptides on CBA/CaJ mice response to Mls-1^a (CBA/J splenocytes), after 8 days (Experiment 1) or after 40 days (Experiment 2).
T cells were purified from popliteal lymph nodes of CBA/CaJ mice immunized with saline (■), Vβ6(48-75) (▨), or Vβ6(58-75) (▩) emulsified in CFA. T cells were cultured at 0.25 x 10^5 (a - c), 0.5 x 10^5 (d - f), 1.0 x 10^5 (g - i) or 2.0 x 10^5 (j - l) cells with 1 x 10^6 (a, d, g, j), 0.5 x 10^6 (b, e, h, k), or 0.25 x 10^6 (c, f, i, l) CBA/J splenocytes in 200 µL culture medium. Incorporation of [^{3}H]dThd was measured as described in Materials and Methods, and each cpm value was converted to the percent of the saline/CFA control response to 1 x 10^6 APC. (* indicates $p < 0.05$, and ** indicates $p < 0.01$ by analysis of variance)

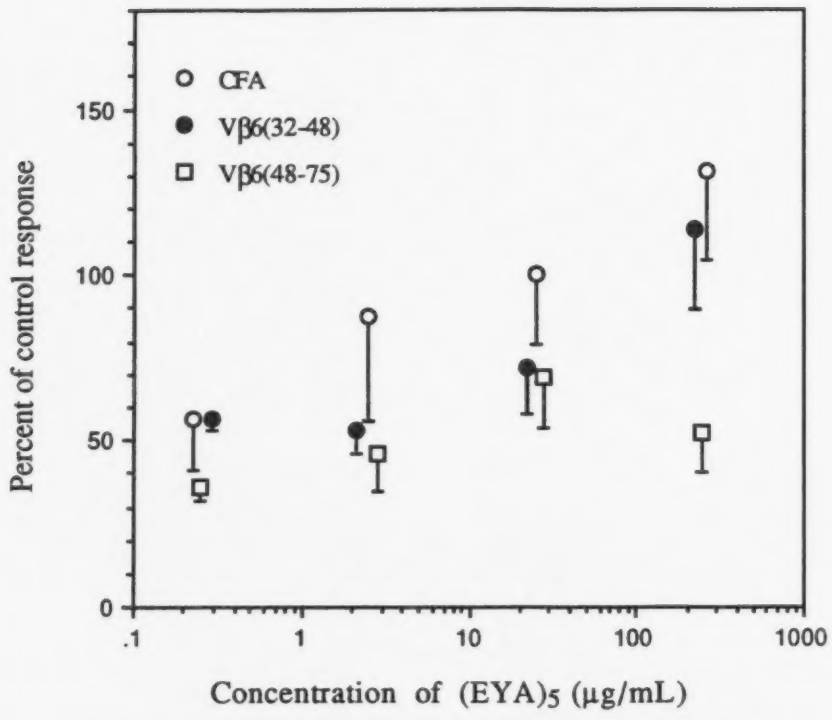

Figure 4.3. Effect of pre-immunization with Vβ6 peptides on the T cell response by BALB/c mice to $(EYA)_5$.
BALB/c mice were immunized with Vβ6(32-48) or Vβ6(48-75) or saline emulsified in CFA, then immunized with $(EYA)_5$ emulsified in IFA, 8 days later. Purified cells from the popliteal lymph nodes were cultured at 4 x 10^5 cells/well with 1 x 10^6 APC and different doses of $(EYA)_5$. Incorporation of $[^3H]$dThd was measured after 4 days and values were converted to the percent of the CFA control response to 25 μg/mL $(EYA)_5$.

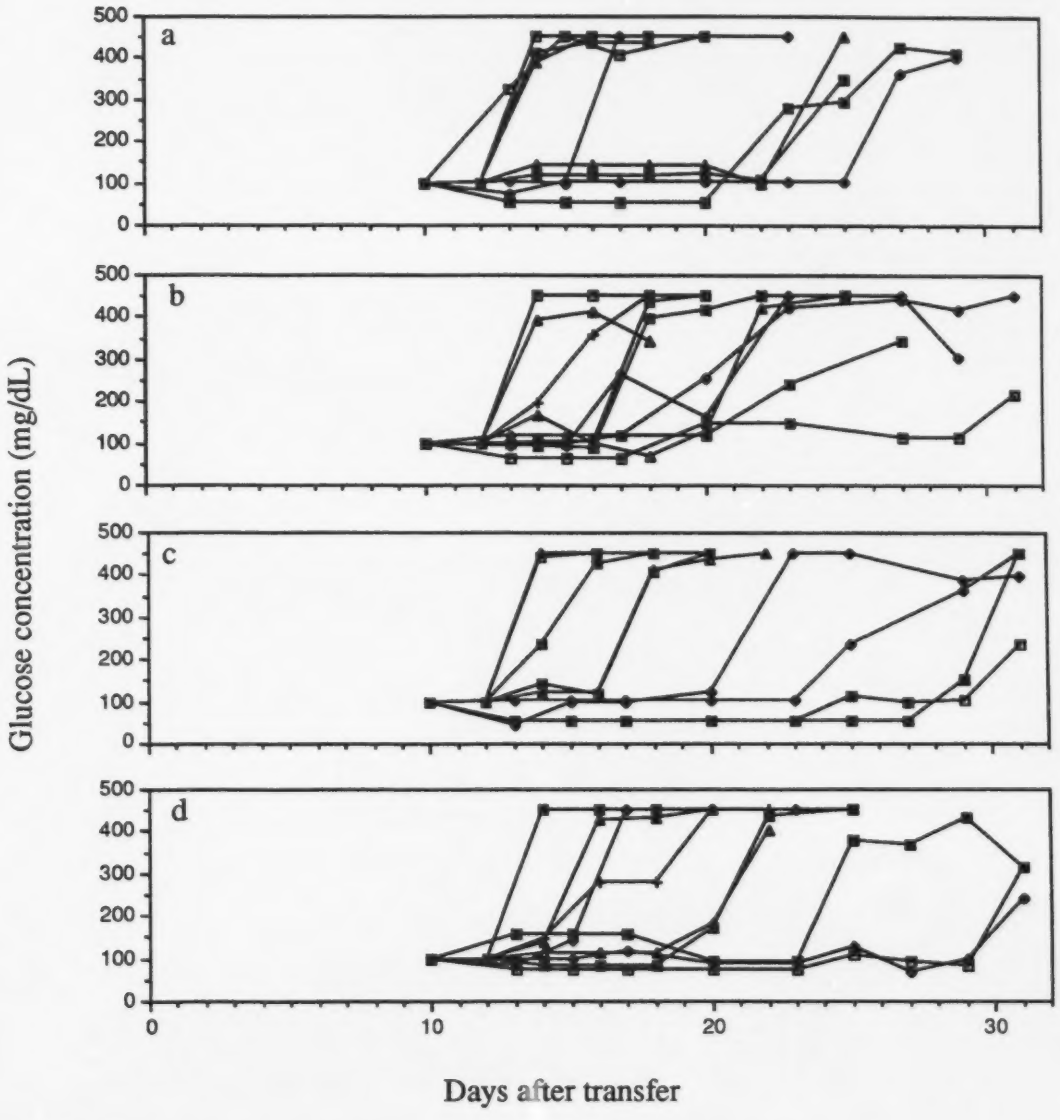

Figure 4.4. Effect of splenocytes from NOD mice primed with Vβ3 peptides on the transfer of diabetes in NOD mice.
Recipient NOD mice were irradiated and given 15 x 10^6 diabetic spleen cells only (a), or 15 x 10^6 diabetic spleen cells mixed with 15 x 10^6 splenocytes from normal NOD primed with saline emulsified in IFA (b), Vβ3(1-20) emulsified in IFA (c), or Vβ3(38-59) emulsified in IFA (d).

Bibliography

1. Jerne, N.K. 1974. Towards a network theory of the immune system. Ann. Immunol. (Inst. Pasteur) 125C:373-389.

2. Ben-Nun, A., H. Wekerle, and I.R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. Nature 292:60-61.

3. Lohse, A.W., E. Mor, T. Reshef, K.-H.M. zum Buschenfelde, and I.R. Cohen. 1990. Inhibition of the mixed lymphocyte reaction by T cell vaccination. Eur. J. Immunol. 20:2521-2524.

4. Binz, H., and H. Wigzell. 1978. Induction of specific immune unresponsiveness with purified mixed leukocyte culture-activated T lymphoblasts as autoimmunogen. III. Proof for the existence of autoanti-idiotypic killer T cells and transfer of suppression to normal syngeneic recipients by T or B lymphocytes. J. Exp. Med. 147:63-76.

5. Lider, O., T. Reshef, E. Beraud, A. Ben-Nun, and I.R. Cohen. 1988. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science 239:181-183.

6. Kourilsky, P., G. Chaouat, C. Rabourdin-Combe, and J.-M. Claverie. 1987. Working principles in the immune system implied by the "peptidic self" model. Proc. Natl. Acad. Sci. USA 84:3400-3404.

7. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541.

8. Offner, H., G.A. Hashim, and A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. Science 251:430-432.

9. Burns, F.R., X. Li, N. Shen, H. Offner, Y.K. Chou, A.A. Vandenbark, and E. Herber-Katz. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. J. Exp. Med. 169:27-30.

10. Kawano, Y.-I., Y. Sasamoto, S. Kotake, S.R. Thurau, B. Wiggert, and I. Gery. 1991. Trials of vaccination against experimental autoimmune uveoretinitis with a T-cell receptor peptide. Curr. Eye Res. 10:789-795.

11. Desquenne-Clark, L., T.R. Esch, L. Otvos, Jr., and E. Heber-Katz. 1991. T-cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis. Proc. Natl. Acad. Sci. USA 88:7219.

12. Kilgannon, P. 1991. T cell receptor repertoire to poly-18 and Mls-1^a: parallels between a peptide antigen and superantigen recognition. PhD Thesis. Univ. of Alberta, Edmonton, Alberta.

13. Webb, S.R., and J. Sprent. 1990. Response of mature unprimed $CD8^+$ T cells to Mls^a determinants. J. Exp. Med. 171:953-958.

14. MacNeil, D., E. Fraga, and B. Singh. Characterization of murine T cell response to peptides of self T cell receptor β chains. (submitted).

15. Sadelain, M.W.J., H.-Y. Qin, J. Lauzon, and B. Singh. 1990. Prevention of type-1 diabetes in NOD mice by adjuvant immunotherapy. Diabetes 39:583-589.

16. Qin, H.-Y., M.W.J. Sadelain, C. Hitchon, J. Lauzon, and B. Singh. Complete Freund's adjuvant induced T cells prevent the development and adoptive transfer of diabetes in nonobese diabetic (NOD) mice. (submitted).

17. Candeias, S., J. Katz, C. Benoist, D. Mathis, and K. Haskins. 1991. Islet-specific T-cell clones from nonobese diabetic mice express heterogeneous T-cell receptors. Proc. Natl. Acad. Sci. USA 88:6167-6170.

18. Nakano, N., H. Kikutani, H. Nishimoto, and T. Kishimoto. 1991. T cells receptor V gene usage of islet β cells-reactive T cells is not restricted in non-obese diabetic mice. J. Exp. Med. 173:1091-1097.

19. Danska, J. 1992. Molecular analysis of T cell receptors resident in the early pancratic infiltates of NOD mice. Annual Meeting of the Canadain Society of Immunologists. Abstract 1.4

20. Fairchild, S., A.M. Knight, P.J. Dyson, and K. Tomaonari. 1991. Co-segregation of a gene encoding a deletion ligand for Tcrβ-V3$^+$ T cells with Mtv-3. Immunogenetics 34:227-230.

21. MacNeil, D., E. Fraga, and B. Singh. 1992. Inhibition of superantigen recognition by peptides of the variable region of the T cell receptor β chain. Eur. J. Immunol. 22:937-941.

22. Lee, K.C., B. Singh, M.A. Barton, A. Proyshyn, and M. Wong. 1979. A simple reliable system for studying antigen-specific murine T cell proliferation. J. Immunol. Methods 25:159.

23. Rohlf, F.J, and R.R. Sokal. 1981. Biometry: The Principles and Practice of Statistics in Biological Research. 2nd ed. W.H. Freeman and Co. (San Francisco).

24. Miller, B.J., M.C. Appel, J.J. O'Neil, and L.S. Wicker. 1988. Both the Lyt-2$^+$ and L3T4$^+$ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. J. Immunol. 140:52-58.

25. Abe, R., M.S. Vacchio, B. Fox, and R.J. Hodes. 1988. Preferential expression of the T-cell receptor Vβ3 gene by Mls^c reactive T cells. Nature 335:827-828.

26. MacDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha-Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. Nature 332:40-45.

27. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature 332:35-40.

28. Happ, M.P., D.L. Woodland, and E. Palmer. 1989. A third T-cell receptor β-chain variable region gene encodes reactivity to Mls-1^a gene products. Proc. Natn. Acad Sci U.S.A. 86:6293-6296.

29. Kronenberg, M. 1991. Self-tolerance and autoimmunity: meeting review. Cell 65:537-542.

30. Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman, and S. Abrignani. 1988. T cells can present antigens such as HIV gp120 targeted to their own surface molecules. Nature 334:530-532.

31. Patten, P., T. Yokota, J. Rothbard, Y.-H. Chien, K.-I. Arai, M.M. Davis. 1984. Structure, expression and divergence of T-cell receptor b-chain variable regions. Nature 312: 40-46.

Chapter V

General discussion and future prospects

In this thesis we have investigated the regulatory role of peptides of the variable region of the TCR in immune responses. Our objectives were to study the immunogenicity of Vβ-peptides and to determine their effect on T cell responses *in vitro* and *in vivo*. First, we tested the *in vitro* effect of Vβ6-peptides on the response of Vβ6$^+$ T cell hybridomas to Mls-1^a bearing APC. Second, we tested the immunogenicity of Vβ-peptides in mice, exploring the influence of MHC and Mls genotypes on responsiveness, in order to identify potentially regulatory peptides. Lastly we tested the *in vivo* effect of immunization with Vβ6-peptides on T cell responses to a superantigen, Mls-1^a, and to a peptide antigen, $(EYA)_5$. In addition, we examined the effect of immunization with Vβ3-peptides on the transfer of diabetes in NOD mice. The results of each of these studies will be summarized and discussed below.

In vitro inhibition by Vβ6-peptides

We observed that several peptides of the Vβ6 gene segment, Vβ6(1-20), Vβ6(48-75), and Vβ6(58-75), could partially inhibit the response of Vβ6$^+$ T cell hybridomas to irradiated Mls-1^{a+} APC, but not to $(EYA)_5$ presented by irradiated Mls-1^{a-} APC. The same

peptides had no effect on the response of a Vβ8$^+$ T cell hybridoma to Mls-1^{a+} APC. Other peptides, Vβ6(32-48) and Vβ6(39-60), had no effect on recognition of superantigen and so served as control peptides. From this one can infer that the sequence of the peptide is important in determining the effect of the Vβ6-peptides. The sequences of the inhibitory peptides indicate that at least amino acids 1 to 20 and 58 to 75 interact with Mls-1^a. The position of these residues corresponds to a solvent-exposed portion of the β-pleated sheet of the Vβ domain, which lies outside the putative antigen binding domain (1). This conclusion is in agreement with other studies using different methods to identify the site on the TCR-Vβ13.2 (2), Vβ8.2 (3), or Vβ8.1 (4) which interacts with superantigen. Our results suggest that Vβ6 peptides can interact with Mls-1^a on the surface of APC and thus compete with the TCR for binding to Mls/MHC complexes. Inhibition was never 100%, even at 75 μM of competing peptide. This indicates that the TCR is more efficient at binding to Mls-1^a than Vβ6-peptides, probably because the Vβ6 peptides are unable to form the tertiary structure of the corresponding region on the TCR. This study demonstrates that Vβ-peptides can inhibit TCR recognition of superantigen.

Immunogenicity of Vβ-peptides

We observed that most of the Vβ-peptides tested are immunogenic in several strains of mice. Responsiveness correlates with MHC haplotype more than with Mls phenotype. This indicates a strong Ir effect with little influence by Mls and the deletion of T cells

expressing TCR with the corresponding Vβ. The T cell response to Vβ6 peptides is mainly by $CD4^+$, class II-restricted T cells, as demonstrated by mAb blocking of the *in vitro* proliferation. Our results show that lack of T cell tolerance to self antigens extends to some epitopes of the TCR. This indicates that T cells reactive to self-peptides can escape thymic deletion, perhaps due to low affinity receptors or the expression of these peptides at densities in sufficient to induce tolerance. Also, the lack of tolerance to Vβ-peptides in spite of the expression of Vβ6 by T cells, is consistent with a prediction of the network theory. Immune recognition of the peptides of the TCR (and Ig) is a necessary requirement for these peptides to have any influence on the immune system.

The induction of Ab by several of the Vβ6-peptides in BALB/c mice was also examined. After two immunizations, Ab response to Vβ6(1-20) and Vβ6(39-60), but not to Vβ6(32-48), was observed. This was a curious result, considering the strong proliferative T cell response to Vβ6(32-48) but not to Vβ6(39-60). We were prompted to examine the cytokine produced by the respective T cell populations.

Two subsets of T helper cells have been identified, Th1 type and Th2 type, which differ in cytokine production and T helper function (5). TH1 type T cells help delayed type hypersensitivity (DTH) reactions and, variably, B cells responses, whereas TH2 type T cells

help B cell responses but not DTH reactions. Consistent with Vβ6(32-48) inducing TH1 type T cells, culture supernatants of primed T cells stimulated with Vβ6(32-48), but not with Vβ6(39-60), contained IL-2 and IFNγ. IL-4 could not be detected in culture supernatants. This was probably due to the insensitivity of the bioassay for IL-4. Others have also observed that different peptides of the same molecule can induce antibody, but not DTH responses, and *vice versa* (6). In that study, the proliferative response to the peptides *in vitro* correlated to the Ab response, not the DTH response. It is therefore unexpected that Vβ6(39-60), which induces an Ab response, can not induce T cell proliferation *in vitro*.

It was also determined that all Vβ6-peptides tested could compete with a peptide antigen, K4, for presentation to T cell hybridomas by syngeneic fixed APC. This demonstrated that Vβ6-peptides, like many other self peptides, can bind to syngeneic MHC molecules (7 - 9). It should be noted here that the results of two antigen competition assays, one in Chapter II and one in Chapter III, differed probably because of different experimental procedures. The two assays differed in the T cell hybridoma, peptide antigen, and APC used. The most important factor in the competition assays is probably the antigen; $(EYA)_5$ may have a much greater affinity for I-A^d than the Vβ6-peptides and so the presentation of $(EYA)_5$ could not be inhibited.

In summary, this study demonstrated the immune response to Vβ6-peptides, indicating that Vβ-peptides have a potential regulatory function *in vivo*.

In vivo regulation by Vβ6-peptides

We observed that immunization with some Vβ6-peptides resulted in a small reduction in the proliferative response to Mls-1^a and to $(EYA)_5$, both of which are dominated by $V\beta6^+$ T cells (10, 11). The reduction in proliferation was not 100% nor observed at all densities of lymphocytes or concentrations of antigen tested. This is likely due to the heterogeneity of the T cell response to both antigens (11, 12). The fact that any reduction in proliferation was observed indicates that some portion of the responding T cells were affected and that immunization with Vβ-peptides can modulate heterogeneous T cell responses. These findings are consistent with other studies of Vβ-peptides and homogeneous T cell responses, such as ablation of the T cell response by mice to myoglobin (13), or protection from EAE in rats (14, 15) by immunization with a Vβ8.2-peptide. However, other investigators have shown that the Vβ8.2-peptide may not consistently protect rats from EAE (16, 17). The failure to induce protection may be due to differences in prior immunological experiences of the population of rats used in those studies. Since the immune network may be influenced by previous immune reactions, modulation with a Vβ-peptide may not produce the same result.

How Vβ6-peptides regulate T cell responses remains to be determined. It is unlikely that antibodies specific for these peptides influence the response of $V\beta6^+$ T cells because (1) few antibodies are produced within 8 days after a single priming event, and (2) antibodies specific for these TCR-peptides do not recognize intact TCR molecules (Fig. 3.1a, Chapter III). In this system therefore, the mechanism by which Vβ-peptides regulate immune responses must be through the induction of T cells which recognize fragments of the Vβ region of the TCR. These T cells, by definition, are anti-idiotypic, which may influence the proliferation and/or function of other T cells expressing the idiotype (18 - 20). The anti-idiotypic T cells may be class II-restricted in those species which have class II^+ T cells, but in mice, which have class II^- T cells, these anti-idiotypic T cells must be class I-restricted. Thus the $CD4^+$, class II-restricted T cells which we observe in response to the Vβ6-peptides are likely not responsible for the reduction in T cell responses to Mls-1^a or $(EYA)_5$. However, a small portion of the T cells which respond to Vβ6-peptides may be class I-restricted. The response to Vβ6(48-75) by CBA/CaJ mice was partially inhibited by anti-K^k mAb (Fig. 3.5b), and, in one experiment, anti-CD8 mAb partially inhibited responses to Vβ6(1-20) by BALB/c mice and to Vβ6(48-75) by CBA/CaJ mice (unpublished observations). We attempted to induce class I-restricted CTL by *in vitro* stimulation with several of the Vβ6-peptides. No lytic activity was observed even after several weeks of re-stimulation with Vβ6-peptides. It may be that CTL specific for Vβ6-peptides are difficult

to clone and other methods are required to induce and propagate these cells *in vitro*. The putative $CD8^+$, class I-restricted T cells, which are specific for Vβ6-peptides, may recognize $V\beta6^+$ T cells and inhibit their response to antigen by cytolysis or the secretion of cytokines.

It is unlikely that T cells specific for Vβ6-peptides are interacting with intact $V\beta6^+$ TCR molecules due to the nature of antigen recognition by the TCR. Thus the anti-idiotypic T cells must recognize fragments of the TCR, which are processed and presented by the target T cell. These processed fragments probably share epitopes with the peptides used to induce the anti-idiotypic T cells. It is interesting to note that the peptides which have some inhibitory effect on the recognition of superantigen or peptide antigen, Vβ6(1-20), Vβ6(48-75) and Vβ6(58-75), are peptides of the solvent exposed face of the Vβ domain (1). One possible explanation for this is that solvent exposed regions of the TCR molecule are presented by T cells at greater densities on the cell surface than are peptides from other regions of the TCR, however, presently there is no evidence to support this supposition.

Unlike the studies on EAE in rats, we were unable to show any protective effect from immunization with Vβ3-peptides on the induction of diabetes in NOD mice. This may be due to the heterogeneous nature of the T cell population which induces diabetes. We chose peptides of Vβ3 because there is some indirect

evidence that Vβ3+ T cells mediate the disease (21), but many Vβ gene segments are expressed among the lymphocytes which infiltrate pancreatic islets (21, 22). It is unknown if all or only a subset of the infiltrating lymphocytes actually induce the onset of insulitis.

Future prospects

This study has only begun to examine the regulatory role of Vβ-peptides in the immune response. Many questions concerning the mechanism of regulation by Vβ-peptides remain to be answered. Do Vβ6 peptides directly bind to Mls/MHC complexes? This probable mechanism of action could be examined with direct *in vitro* binding assays. Inhibiting peptides would be expected to bind to Mls/MHC complexes with greater affinities than the non-inhibitory peptides.

Is the reduction in proliferative response to Mls-1^a and $(EYA)_5$ due to the loss of reactivity of Vβ6+ T cells, as proposed? The frequency of Vβ6+ T cells after 4d stimulation with Vβ6(1-20) or Vβ6(48-75) is not altered compared to T cells stimulated with an irrelevant antigen (Table 3.3). Thus it is unlikely that the Vβ6+ T cells are lysed by cytotoxic cells, rather they may be suppressed or rendered unresponsive. To test this, the expression of Vβ6+ T cells could be measured in different groups after stimulation with Mls-1^a or $(EYA)_5$. The frequency of Vβ6+ blast cells in the peptide immunized groups may be lower than that in the saline/CFA group, which

would exhibit an expanded population of Vβ6$^+$ T cells due to the stimulation by Mls-1^a or $(EYA)_5$.

What class of Vβ-specific T cells regulate T cell responses? The procedure of pre-immunization with Vβ-peptides before stimulation of antigen does not identify the regulatory cell involved. Mixing and transfer experiments are required to characterize the type of T cell which inhibits the proliferation or induction of the antigen reactive T cells. As discussed above, the regulatory T cell is likely to be CD8$^+$, but there is no direct evidence that this is the case in this system.

Are there CD8$^+$, class-I restricted T cells which recognize Vβ-peptides? The T cell response to Vβ-peptides may have been biased toward CD4$^+$ T cells by the method of immunization used in this study. It should be possible to induce CD8$^+$ T cells by appropriate priming methods, such as intraperitoneal injection of cell-associated peptides. Also, since CD8$^+$ T cells tend to recognize peptides of about 8 amino acids in length, shorter peptides containing class I-binding motifs may be more appropriate to use in priming and *in vitro* testing of the class I-restricted T cells.

Can CD8$^+$ T cells specific for Vβ-peptides regulate other T cells? As discussed above, it is unlikely that the regulatory T cells induced by Vβ-peptides lyse the Vβ6$^+$ T cells. However, it is possible that class-I restricted T cells may inhibit the response of Vβ6$^+$ T cells by

other means, for example by cytokines. If class I-restricted T cells specific for Vβ6-peptides could be generated, it would be interesting to test their effect on the response of Vβ6$^+$ T cells *in vitro*. If no direct lysis can be demonstrated, then the cytokines released by these Vβ6 specific T cells could be determined.

What is the epitope recognized by the regulating T cells? This may be approached by identifying the minimal Vβ6-peptides sufficient to induce the regulatory effect or to stimulate regulatory T cell clones *in vitro*. In addition the endogenous peptides which bind to the MHC molecules of Vβ6$^+$ T cells and stimulate regulatory T cells could be identified following acid elution from purified class I or class II molecules. The eluant could then be separated by high pressure liquid chromatography, and the fractions assayed in a bioassy for their ability to stimulate T cells specific for Vβ6-peptides. Active fractions could be microsequenced to determine the stimulatory peptide(s) primary structure.

In conclusion, we have extended the evidence for Vβ-peptides in regulating T cell responses. *In vitro*, Vβ-peptides can directly inhibit T cell recognition of superantigen, and delineate the site of recognition of TCR for superantigen. Consistent with the network theory, peptides of the variable region of TCR are immunogenic, and can modulate T cell proliferative responses to the superantigen, Mls-1^a, and a peptide antigen $(EYA)_5$.

Bibliography

1. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of the T cell αβ receptor. EMBO J. 7:3745-3755.

2. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T-cell-receptor β-chain that interact with *S. aureus* toxin superantigens. Nature 346:471-473.

3. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor β chain that interacts with the self-superantigen Mls-1^a. Cell 61:1365-1374.

4. Pullen, A.M., J. Bill, R.T. Kubo, P. Marrack, and J.W. Kappler. 1991. Analysis of the interaction site for the self superantigen Mls-1^a on T cell receptor Vβ. J. Exp. Med. 173:1183-1192.

5. Mosmann, T.R., and R.L. Coffman. 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann. Rev. Immunol. 7:145-173.

6. Ashbridge, K.R., B.T. Backstrom, H.-X. Li, T. Vikerfors, D.R. Englebretsen, D.R.K. Harding, and J.D. Watson. 1992. Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of *Mycobacterium tuberculosis*: evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity responses. J. Immunol. 148:2248-2255.

7. Schild, H., O. Fotzschke, H. Kalbacher, and H.-G. Rammensee. 1990. Limit of T cell tolerance to self proteins by peptide presentation. Science 247:1587.

8. Benichou, G., P.A. Takizawa, P.T. Ho, C.C. Killion, C.A. Olson, M. McMillan, and E.E. Sercarz. 1990. Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides. J. Exp. Med. 172:1341.

9. Agrawal, B., M. Manickasundari, E. Fraga, and B. Singh. 1991. T cells that recognize peptide sequences of self MHC class II molecules exist in syngeneic mice. J. Immunol. 147:383.

10. Webb, S.R., and J. Sprent. 1990. Response of mature unprimed $CD8^+$ T cells to Mls^a determinants. J. Exp. Med. 171:953-958.

11. Kilgannon, P. 1991. T cell receptor repertoire to poly-18 and Mls-1a: parallels between a peptide antigen and superantigen recognition. PhD Thesis. Univ. of Alberta, Edmonton, Alberta.

12. Herman, A., J.W. Kappler, P. Marrack, and A.M. Pullen. 1991. Superantigens: mechanism of T-cell stimulation and role in immune responses. Annu. Rev. Immunol. 9:74-772.

13. Kronenberg, M. 1991. Self-tolerance and autoimmunity: meeting review. Cell 65:537-542.

14. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541.

15. Offner, H., G.A. Hashim, and A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. Science 251:430-432.

16. Kawano, Y.-I., Y. Sasamoto, S. Kotake, S.R. Thurau, B. Wiggert, and I. Gery. 1991. Trials of vaccination against experimental autoimmune uveoretinitis with a T-cell receptor peptide. Curr. Eye Res. 10:789.

17. Desquenne-Clark, L., T.R. Esch, L. Otvos, Jr., and E. Heber-Katz. 1991. T-cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis. Proc. Natl. Acad. Sci. USA 88:7219.

18. Lohse, A.W., E. Mor, T. Reshef, K.-H.M. zum Buschenfelde, and I.R. Cohen. 1990. Inhibition of the mixed lymphocyte

reaction by T cell vaccination. Eur. J. Immunol. 20:2521-2524.

19. Heuer, J., and E. Kolsch. 1986. Selective elimination through a cytolytic mechanism of bovine serum albumin-specific T helper lymphocytes by T suppressor cells with the same antigen specificity. Eur. J. Immunol. 16:400-404.

20. Sun, D., Y. Qin, J. Chluba, J.T. Epplen, and H. Wekerle. 1988. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interaction. Nature 332:843-845.

21. Candeias, S., J. Katz, C. Benoist, D. Mathis, and K. Haskins. 1991. Islet-specific T-cell clones from nonobese diabetic mice express heterogeneous T-cell receptors. Proc. Natl. Acad. Sci. USA 88:6167-6170.

22. Nakano, N., H. Kikutani, H. Nishimoto, and T. Kishimoto. 1991. T cells receptor V gene usage of islet β cells-reactive T cells is not restricted in non-obese diabetic mice. J. Exp. Med. 173:1091-1097.